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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIVI-GAG. POL. NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1- Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.



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A. CLAS	SSIFICATION OF SUBJECT MATTER						
IPC(7) : C12N 15/86							
US CL : 435/456							
According to International Patent Classification (IPC) or to both national classification and IPC  B. FIELDS SEARCHED							
		l bu alamaici	antine symbols)				
U.S. : 4	cumentation searched (classification system followed 24/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.	.3, 235.1, 3	20.1, 456; 530/23.72;				
Documentation	on searched other than minimum documentation to th	ne extent tha	t such documents are include	d in the fields searched			
	ata base consulted during the international search (nar continuation Sheet	me of data t	ease and, where practicable, s	earch terms used)			
C. DOC	UMENTS CONSIDERED TO BE RELEVANT						
Category *	Citation of document, with indication, where a	ppropriate,	of the relevant passages	Relevant to claim No.			
X	WO 96/39178 (ERTL et al.) 12 December 1996 (12	2.12.1996),	see page 5, 6,10, 12, 13	1-3, 8-11, 18			
	and claims 1 and 5.	•					
Y				4, 5, 13-17, 29, 30, 32, 34, 35, 37			
` <b>x</b>	US 6,019,978 A (ERTL et al.) 1 February 2000 (01	1/02/2000),	see columns 2, 7 and 8.	1-3, 8-11, 18			
Y				4, 5, 13-17, 29, 30, 32, 34, 35, 37			
X,P	US 6,287,571 A A (ERTL et al.) 11 September 2001 (11/09/2001), see columns 2, 7, 8			1, 9, 18			
x	and claim 1. US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/	1997), see e	examples 1, 2, 25 and 26.	1-3, 8, 9-11, 18			
Y			•	4,5,13-17, 29, 30, 32, 34, 35, 37			
Y	WANG et al. The use of an E1-deleted, replication expressing the rabies virus glycoprotein for early vi Journal of Virology (March 1997) Vol. 71, No. 5, p	accination o	f mice against rabies virus.	1-3, 9-11, 13-18			
Further	documents are listed in the continuation of Box C.		See patent family annex.				
"A" document	pecial categories of cited documents: defining the general state of the art which is not considered to be	-T-	later document published after the inte date and not in conflict with the applic principle or theory underlying the inve	ation but cited to understand the			
•	of particular relevance  X*  document of particular relevance; the claimed invention cannot be earlier application or patent published on or after the international filing date  Considered novel or cannot be considered to involve an inventive step						
establish	establish the publication date of another citation or other special reason (as "Y" document of particular relevance; the claimed invention cannot be						
	specified) considered to involve an inventive step when the document is combined with one or more other such documents, such combination of document referring to an oral disclosure, use, exhibition or other means being obvious to a person skilled in the art						
	"P" document published prior to the international filing date but later than the "&" document member of the same patent family priority date claimed						
Date of the a	Date of the actual completion of the international search  Date of mailing of the international search report  13 MAR 2002			rch report			
	2002 (06.02.2002)			-A-			
	Name and mailing address of the ISA/US  Authorized officer						
			inkler, Ph.D.	1/1/11			
	Washington, D.C. 20231 Facsimile No. (703)305-3230 Telephone No. 703-308-0196						
	A/210 (second sheet) (July 1998)	<u> </u>		<del></del>			
, 0,111. 0 1/10.	(			A			

International application No.

#### PCT/US01/28861

### INTERNATIONAL SEARCH REPORT

Citation of document, with indication, where appropriate of the relevant passages  NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses	1, 9, 29, 30, 32
(1993) Vol. 9, No. 5, pp395-404, see material and methods.	
PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficincy Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29, 30, 32
LORI et al. Rapid protection against human immunodeficiency virus type ! (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1, 9
PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	16
NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	1,9
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	Deficincy Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.  LORI et al. Rapid protection against human immunodeficiency virus type ! (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.  PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp. 115-22, see abstract.  NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.

International application No.

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Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)		
This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:		
Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely:		
2. Claim Nos.: 31 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  This claim could not be searched because applicant did not provide a CRF.		
3. Claim Nos.:  because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).		
Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)		
This International Searching Authority found multiple inventions in this international application, as follows: Please See Continuation Sheet		
1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.		
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.		
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:		
No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37		
Remark on Protest  The additional search fees were accompanied by the applicant's protest.  No protest accompanied the payment of additional search fees.		
110 protest accompanies the payment of auditorial seaten rees.		

Form PCT/ISA/210 (continuation of first sheet(1)) (July 1998)

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The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences a encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

#### Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, STN-BIOSIS, MEDLINE adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter

International application No.

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	<del> </del>	
		and AE3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ 1D NO: 5) inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immane response to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
19	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in the parallel orientation of E1.
20	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of ΔΕ1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the parallel orientation of Ε1.
21	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the parallel orientation of E1.
22	67-70, 72, 73, 75	The claims are directed to an adenoviral vector that is at least partially deleted of AE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type

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		the state of the s
		and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type
	1	adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1)
		inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
	1	and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type
	ı	adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5)
	1	inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
13	1 22	and AE3 the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7)
		inserted in E1.
		The claims are directed to a method of making and harvesting of a recombinant
16	57-61	adenoviral particle that contains a gene encoding an HIV Pol protein.
		adenoviral particle that contains a gene encoding air triviror protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response
		to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response
	1	to HIV Pol protein with the recombinant adenoviral particle in addition to
	1	administering a DNA plasmid vaccine.
19	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	AE1, the vector contains the cis-acting packaging sequence of the wild type
	1	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
	1	inserted in the parallel orientation of E1.
20	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
20		ΔΕ1, the vector contains the cis-acting packaging sequence of the wild type
	73, 75	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
	{	inserted in the parallel orientation of E1.
	<del>                                     </del>	The claims are directed to an adenoviral vector that is at least partially deleted of
21	67-70, 72,	The claims are directed to an authorital vector that is at least partially defeted of
	73, 75	ΔE1, the vector contains the cis-acting packaging sequence of the wild type
	1	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)
		inserted in the parallel orientation of E1.
22	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	ΔE1, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15)
		inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ .
	1"	the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in
		the antiparallel orientation of E1.
	<del>- </del>	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ .
24	71	the vector contains the cis-acting packaging sequence of the wild type adenovirus
	į.	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in
		the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ ,
	ļ	the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in
	l	the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E_1$ ,
		the vector contains the cis-acting packaging sequence of the wild type adenovirus
	1	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in
	l l	the antiparallel orientation of E1.
27		The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta EI$
27	74	and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type
		and AES, the vector contains the cis-acting packaging sequence of the whotype adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
		inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E_1$
		and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
		inserted in E1.
		The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
29	74	I THE CISIM IS DIRECTED IN AN AUCTIONIST ACCION THE 12 BY 16821 DOI 11811 ACCION OF 1882

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		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)
		inserted in E1.
30	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E_1$
-		and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type
	ļ	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15)
		inserted in E1.
31	76-80	The claims are directed to a method of making and harvesting of a recombinant
31		adenoviral particle that contains a gene encoding an HIV Nef protein.
20	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune
32	"" '	response to HIV Nef with the recombinant adenoviral particle.
	82, 83	The claims are directed to a method of generating a cellular mediated immune
33	02, 00	response to HIV Nef with the recombinant adenoviral particle in addition to
	ł	administering a DNA plasmid vaccine.
	86a	The claim is drawn to a multivalent vaccine wherein gag, pol and nef are expressed
34	802	from three individual vectors.
		The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
35	86b, 88, 89	from one individual vectors.
		The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
36	86c, 88	from two individual vectors, one expressing nef-pol fusion and one expressing gag.
		The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
37	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein gag, pot and met are expression and
		from two individual vectors, one expressing gag-pol fusion and one expressing nef.
38	86e, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
50		from two individual vectors, one expressing nef-gag fusion and one expressing pol.
39	86f, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed
39		from a single vectors as a fusion protein.
40	86g, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed
40	008,	from two individual vectors.
	86h, 88, 89	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed
41	8011, 55, 55	individually from one vector.
	86i, 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed
42	801, 60	from two individual vectors.
	06: 00 90	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed
43	86j, 88, 89	from individually from one vector.
		The claims are drawn to a multivalent vaccine wherein nef and gag are expressed
44	86k, 88	individually from one vector.
		The claims are drawn to a multivalent vaccine wherein nef and gag are expressed
45	861, 88, 89	
		individually from one vector.
46	86m, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed as a
	1	fusion protein from one vector.
47	86n, 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed as a
		fusion protein from one vector.
		The claims are drawn to a multivalent vaccine wherein nef and gag are expressed as a
48	860, 88	The clause are drawn to a material and the clause and are of the clause and the clause are of the clause and the clause are of the clause

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Erd et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

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(71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

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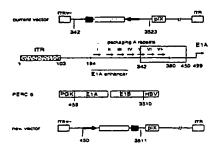
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(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS



(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HTV1- Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.





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#### TITLE OF THE INVENTION

ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

#### 5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S. provisional applications 60/233,180, 60/279,056, and Attorney Docket 20867PV2 (serial number unassigned), filed September 15, 2000, March 27, 2001, and September 7, 2001, respectively.

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# STATEMENT REGARDING FEDERALLY-SPONSORED R&D Not Applicable

#### REFERENCE TO MICROFICHE APPENDIX

15 Not Applicable

#### FIELD OF THE INVENTION

The present invention relates to recombinant, replication-deficient first generation adenovirus vaccines found to exhibit enhanced growth properties and greater cellular-mediated immunity as compared to other replication-deficient vectors. The invention also relates to the associated first generation adenoviral vectors described herein, which, through the incorporation of additional 5' adenovirus sequence, enhance large scale production efficiency of the recombinant, replicationdefective adenovirus described herein. Another aspect of the instant invention is the surprising discovery that the intron A portion of the human cytomegalovirus (hCMV) promoter constitutes a region of instability in adenoviral vector constructs. Removal of this region from adenoviral expression constructs results in greatly improved vector stability. Therefore, improved vectors expressing a transgene under the control of an intron A-deleted CMV promoter constitute a further aspect of this invention. These adenoviral vectors are useful for generating recombinant adenovirus vaccines against human immunodeficiency virus (HIV). In particular, the first generation adenovirus vectors disclosed herein are utilized to construct and generate adenovirus-based HIV-1 vaccines which contain HTV-1 Gag, HIV-1 Pol and/or HIV-1 Nef polynucleotide pharmaceutical products, and biologically active modifications thereof. Host administration of the recombinant, replication-deficient adenovirus vaccines described herein results in expression of HIV-1 Gag, HIV-1- Pol and/or Nef protein or

immunologically relevant modifications thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding codon optimized HIV-1 Gag, HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef, and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The HIV adenovirus vaccines of the present invention, when administered alone or in a combined modality and/or prime/boost regimen, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

#### BACKGROUND OF THE INVENTION

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Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5'LTR-gag-pol-env-LTR 3'organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

The gag gene encodes a 55-kilodalton (kDa) precursor protein (p55) which is expressed from the unspliced viral mRNA and is proteolytically processed by the HIV protease, a product of the pol gene. The mature p55 protein products are p17 (matrix), p24 (capsid), p9 (nucleocapsid) and p6.

The pol gene encodes proteins necessary for virus replication; a reverse transcriptase, a protease, integrase and RNAse H. These viral proteins are expressed as a Gag-Pol fusion protein, a 160 kDa precursor protein which is generated via a ribosomal frame shifting. The viral encoded protease proteolytically cleaves the Pol polypeptide away from the Gag-Pol fusion and further cleaves the Pol polypeptide to the mature proteins which provide protease (Pro, P10), reverse transcriptase (RT, P50), integrase (IN, p31) and RNAse H (RNAse, p15) activities.

The *nef* gene encodes an early accessory HIV protein (Nef) which has been shown to possess several activities such as down regulating CD4 expression, disturbing T-cell activation and stimulating HIV infectivity.

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The env gene encodes the viral envelope glycoprotein that is translated as a 160-kilodalton (kDa) precursor (gp160) and then cleaved by a cellular protease to yield the external 120-kDa envelope glycoprotein (gp120) and the transmembrane 41-kDa envelope glycoprotein (gp41). Gp120 and gp41 remain associated and are displayed on the viral particles and the surface of HIV-infected cells.

The *tat* gene encodes a long form and a short form of the Tat protein, a RNA binding protein which is a transcriptional transactivator essential for HIV-1 replication.

The *rev* gene encodes the 13 kDa Rev protein, a RNA binding protein. The Rev protein binds to a region of the viral RNA termed the Rev response element (RRE). The Rev protein promotes transfer of unspliced viral RNA from the nucleus to the cytoplasm. The Rev protein is required for HIV late gene expression and in turn, HIV replication.

Gp120 binds to the CD4/chemokine receptor present on the surface of helper T-lymphocytes, macrophages and other target cells in addition to other co-receptor molecules. X4 (macrophage tropic) virus show tropism for CD4/CXCR4 complexes while a R5 (T-cell line tropic) virus interacts with a CD4/CCR5 receptor complex. After gp120 binds to CD4, gp41 mediates the fusion event responsible for virus entry. The virus fuses with and enters the target cell, followed by reverse transcription of its single stranded RNA genome into the double-stranded DNA via a RNA dependent DNA polymerase. The viral DNA, known as provirus, enters the cell nucleus, where the viral DNA directs the production of new viral RNA within the nucleus, expression of early and late HIV viral proteins, and subsequently the production and cellular release of new virus particles. Recent advances in the ability to detect viral load within the host shows that the primary infection results in an extremely high generation and tissue distribution of the virus, followed by a steady state level of virus (albeit through a continual viral production and turnover during this phase), leading ultimately to another burst of virus load which leads to the onset of clinical AIDS. Productively infected cells have a half life of several days, whereas chronically or latently infected cells have a 3-week half life, followed by non-productively infected cells which have a long half life (over 100 days) but do not significantly contribute to day to day viral loads seen throughout the course of disease.

Destruction of CD4 helper T lymphocytes, which are critical to immune defense, is a major cause of the progressive immune dysfunction that is the hallmark of HIV infection. The loss of CD4 T-cells seriously impairs the body's ability to fight most invaders, but it has a particularly severe impact on the defenses against viruses, fungi, parasites and certain bacteria, including mycobacteria.

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Effective treatment regimens for HIV-1 infected individuals have become available recently. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. As noted above, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HIV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. CD8+ T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive CD8<sup>+</sup> T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal

induction of CTL responses usually requires "help" in the form of cytokines from CD4<sup>+</sup> T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

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European Patent Applications 0 638 316 (Published February 15, 1995) and 0 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including *env* or *gag*. Various treatment regimens were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

Replication-defective adenoviral vectors harboring deletions in the E1 region are known, and recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging efficiency of adenovirus has been taught to depend on the number of incorporated individual A (packaging) repeats; *see*, *e.g.*, Gräble and Hearing, 1990 *J. Virol.* 64(5):2047-2056; Gräble and Hearing, 1992 *J. Virol.* 66(2):723-731.

Larder, et al., (1987, Nature 327: 716-717) and Larder, et al., (1989, Proc. Natl. Acad. Sci. 86: 4803-4807) disclose site specific mutagenesis of HIV-1 RT and the effect such changes have on *in vitro* activity and infectivity related to interaction with known inhibitors of RT.

Davies, et al. (1991, *Science* 252:, 88-95) disclose the crystal structure of the RNase H domain of HIV-1 Pol.

Schatz, et al. (1989, *FEBS Lett.* 257: 311-314) disclose that mutations Glu478Gln and His539Phe in a complete HIV-1 RT/RNase H DNA fragment results in defective RNase activity without effecting RT activity.

Mizrahi, et al. (1990, *Nucl. Acids. Res.* 18: pp. 5359-5353) disclose additional mutations Asp443Asn and Asp498Asn in the RNase region of the *pol* gene which also results in defective RNase activity. The authors note that the Asp498Asn mutant was difficult to characterize due to instability of this mutant protein.

Leavitt, et al. (1993, *J. Biol. Chem.* 268: 2113-2119) disclose several mutations, including a Asp64Val mutation, which show differing effect on HIV-1 integrase (IN) activity.

Wiskerchen, et al. (1995, J. Virol. 69: 376-386) disclose singe and double mutants, including mutation of aspartic acid residues which effect HIV-1 IN and viral replication functions.

It would be of great import in the battle against AIDS to produce a prophylactic- and/or therapeutic-based HIV vaccine which generates a strong cellular immune response against an HIV infection. The present invention addresses and meets these needs by disclosing a class of adenovirus vaccines which, upon host administration, express codon optimized and modified versions of the HIV-1 genes, gag, pol and nef. These recombinant, replication-defective adenovirus vaccines may be administered to a host, such as a human, alone or as part of a combined modality regimen and/or prime-boost vaccination regimen with components of the present invention and/or a distinct viral HIV DNA vaccine, non-viral HIV DNA vaccine, HIV subunit vaccine, an HIV whole killed vaccine and/or a live attenuated HIV vaccine.

#### SUMMARY OF THE INVENTION

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The present invention relates to enhanced replication-defective recombinant adenovirus vaccine vectors and associated recombinant, replication-deficient adenovirus vaccines which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef. The adenovirus vaccines of the present invention express HIV antigens and provide for improved cellular-mediated immune responses upon host administration. Potential vaccinees include but are not limited to primates and especially humans and non-human primates, and also include any non-human mammal of commercial or domestic veterinary importance. An effect of the improved recombinant adenovirus-based vaccines of the present invention should be a lower transmission rate to previously uninfected individuals (i.e., prophylactic applications) and/or reduction in the levels of the viral loads within an infected individual (i.e., therapeutic applications), so as to prolong the asymptomatic phase of HIV-1 infection. In particular, the present invention relates to adenoviral-based vaccines which encode various forms of codon optimized HIV-1 Gag (including but in no way limited to p55 versions of codon optimized full length (FL) Gag and tPA-Gag fusion proteins), HIV-1 Pol, HIV-1 Nef, and selected modifications of immunological relevance. The administration, intracellular delivery and expression of these adenovirus vaccines elicit a host CTL and Th response. The preferred replication-defective recombinant adenoviral vaccine vectors include but are not limited to synthetic DNA molecules which (1) encode codon optimized versions of wild type HIV-1 Gag; (2) encode codon optimized versions of HIV-1 Pol; (3) encode codon optimized versions of HIV-1 Pol fusion proteins; (4) encode codon optimized versions of modified HIV-1 Pol proteins and fusion proteins, including but not limited

to pol modifications involving residues within the catalytic regions responsible for RT, RNase and IN activity within the host cell; (5) encode codon optimized versions of wild type HIV-1 Nef; (6) codon optimized versions of HIV-1 Nef fusion proteins; and/or (7) codon optimized versions of HIV-1 Nef derivatives, including but not limited to nef modifications involving introduction of an amino-terminal leader sequence, removal of an amino-terminal myristylation site and/or introduction of dileucine motif mutations. The Nef-based fusion and modified proteins, disclosed within this specification and expressed from an adenoviral-based vector vaccine this specification, may possess altered trafficking and/or host cell function while retaining the ability to be properly presented to the host MHC I complex and in turn elicit a host CTL and Th response. Examples of HIV-1 Gag, Pol and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH<sub>2</sub>-teriminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

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The adenoviral vector utilized in construction of the HIV-1 Gag-, HIV-1 Poland/or HIV-1 Nef- based vaccines of the present invention may comprise any replication-defective adenoviral vector which provides for enhanced genetic stability of the recombinant adenoviral genome through large scale production and purification of the recombinant virus. In other words, an HIV-1 Gag-, Pol- or Nef-based adenovirus vaccine of the present invention is a purified recombinant, replicationdefective adenovirus which is shown to be genetically stable through multiple passages in cell culture and remains so during large scale production and purification procedures. Such a recombinant adenovirus vector and harvested adenovirus vaccine lends itself to large scale dose filling and subsequent worldwide distribution procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of a replication-defective adenoviral vector and vectors derived therefrom, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome. A preferred embodiment of the instant invention comprises base pairs 1-450 of a wildtype adenovirus. In other preferred embodiments, the replication -defective adenoviral vector has, in addition thereto, a region 3' to the E1-deleted region comprising base pairs 3511-3523. Basepairs 342-450 (more particularly, 400-450) constitute an extension of the 5'region of previously disclosed vectors carrying viral antigens, particularly HIV antigens (see, e.g., PCT International Application PCT/US00/18332, published

January 11, 2001 (WO 01/02067), which claims priority to U.S. Provisional Application Serial Nos. 60/142,631 and 60/148,981, filed 7/6/1999 and 8/13/1999, respectively; these documents herein incorporated by reference. Applicants have found that extending the 5' region further into the E1 gene into the disclosed vaccine vectors incorporated elements found to be important in optimizing the packaging of the virus.

As compared to previous vectors not comprising basepairs from about 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome, vectors comprising the above region exhibited enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, a more potent virus effect, allowing lower doses of virus to be used to generate equivalent immunity; and a greater cellular-mediated immune response than replication-deficient vectors not comprising this region (basepairs 1-450). Even more important, adenoviral constructs derived therefrom are very stable genetically in large-scale production, particularly those comprising an expression cassette under the control of a hCMV promoter devoid of intron A. This is because Applicants have surprisingly found that the intron A portion of the hCMV promoter constituted a region of instability when employed in adenoviral vectors. Applicants have, therefore, identified an enhanced adenoviral vector which is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

A preferred embodiment of this invention is a replication-defective adenoviral vector in accordance with the above description wherein the gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

In preferred embodiments, the E1 gene, other than that contained within basepairs 1-450 or, alternatively, that contained within base pairs 1-450 and 3511-3523 has been deleted from the adenoviral vector, and the gene expression cassette has replaced the deleted E1 gene. In other preferred embodiments, the replication defective adenovirus genome does not have a functional E3 gene, or the E3 gene has been deleted. Most preferably, the E3 region is present within the adenoviral genome. Further preferred embodiments are wherein the gene expression cassette is in an E1 anti-parallel (transcribed in a 3' to 5' direction relative to the vector backbone)

orientation or, more preferably, an E1 parallel (transcribed in a 5' to 3' direction relative to the vector backbone) orientation.

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Further embodiments relate to a shuttle plasmid vector comprising: an adenoviral portion and a plasmid portion, wherein said adenovirus portion comprises: a) a replication defective adenovirus genome, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) of the wildtype adenovirus genome and, preferably, in addition thereto, basepairs 3511-3523 of a wildtype adenovirus sequence; and b) a gene expression cassette comprising: (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and (c) a transcription terminator and/or a polyadenylation site.

Other aspects of this invention include a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

To this end, the present invention particularly relates to harvested recombinant, replication defective virus derived from a host cell, such as but not limited to 293 cells or PER.C6® cells, including but not limited to harvested virus related to any of the MRKAd5 vector backbones, with or without an accompanying transgene, including but not limited to the HIV-1 antigens described herein. An HIV-1 vaccine is represented by any harvested, recombinant adenovirus material which expresses any one or more of the HIV-1 antigens disclosed herein. This harvested material may then be purified, formulated and stored prior to host administration.

Another aspect of this invention is a method of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising:

a) a recombinant, replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting adenovirus packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) and, preferably in addition thereto, base pairs 3511-3523 of a wildtype adenovirus sequence, and,

b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

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In view of the efficacious nature of the adenoviral and/or DNA plasmid vaccines described herein, the present invention relates to all methodology regarding administration of one or more of these adenoviral and/or DNA plasmid vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. As discussed herein, such a treatment regimen may include a monovalent or multivalent composition, various combined modality applications, and/or a prime/boost regimen to as to optimize antigen expression and a concomitant cellular-mediated and/or humoral immune response upon inoculation into a living vertebrate tissue. Therefore, the present invention provides for methods of using the adenoviral and/or DNA plasmid vaccines disclosed herein within the various parameters disclosed herein as well as any additional parameters known in the art, which, upon introduction into mammalian tissue induces intracellular expression of the gag, pol and/or nef-based vaccines.

To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given more than one administration of adenovirus vaccine vector, and it may be given in a regimen accompanied by the administration of a plasmid vaccine. The plasmid vaccine (also referred to herein as a "DNA plasmid vaccine" or "vaccine plasmid" comprises a nucleic acid encoding a protein or an immunologically relevant portion thereof, a heterologous promoter operably linked to the nucleic acid sequence, and a transcription terminator or a polyadenylation signal (such as bGH or SPA, respectively). There may be a predetermined minimum amount of time separating the administrations. The individual can be given a first dose of plasmid vaccine, and then a second dose of plasmid vaccine. Alternatively, the individual may be given a first dose of adenovirus vaccine, and then a second dose of adenovirus vaccine. In other embodiments, the plasmid vaccine is administered first, followed after a time by administration of the adenovirus vaccine. Conversely, the adenovirus vaccine may be administered first, followed by administration of plasmid vaccine after a time. In these embodiments, an individual may be given multiple doses of the same adenovirus serotype in either viral vector or plasmid form, or the virus may be of

differing serotypes. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

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The present invention also relates to multivalent adenovirus vaccine compositions which comprise Gag, Pol and Nef components described herein; see, e.g., Example 29 and Table 25. Such compositions will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not limitations, include MRKAd5-vector based multivalent vaccine compositions which provide for a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components) composition. Such a mutlivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component; and may in addition be part of a prime/boost regimen with viral or non-viral vector vaccines as introduced in the previous paragraph. To this end, preferred compositions are MRKAd5 adenovirus used in combination with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HIV-1 viral antigens may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example, a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES). Therefore, a multivalent vaccine delivered as a single, or possibly a second harvested recombinant, replication-deficient adenovirus is contemplated as part of the present invention.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

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It is an object of the present invention to provide for enhanced replication-defective recombinant adenoviral vaccine vector backbones. These recombinant adenoviral backbones may accept one or more transgenes, which may be passaged through cell culture for growth, amplification and harvest.

It is a further object to provide for enhanced replication-defective recombinant adenoviral vaccine vectors which encode various transgenes.

It is also an object of the present invention to provide for a harvested recombinant, replication-deficient adenovirus which shows enhanced growth and amplification rates while in combination with increased virus stability after continuous passage in cell culture. Such a recombinant adenovirus is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

To this end, it is an object of the present invention to provide for (1) enhanced replication-defective recombinant adenoviral vaccine vectors as described herein which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag; HIV-1 Pol and HIV-1 Nef, and (2) harvested, purified recombinant replication-deficient adenovirus generated by passage of the adenoviral vectors of (1) through one or multiple passages through cell culture, including but not limited to passage through 293 cells or PER.C6® cells.

It is also an object of the present invention to provide for recombinant adenovirus harvested by one or multiple passages through cell culture. As relating to recombinant adenoviral vaccine vector, this recombinant virus is harvested and formulated for subsequent host administration.

It is also an object of the present invention to provide for replication-defective adenoviral vectors wherein at least one gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

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polyadenylation site.

It is also an object of the present invention to provide for a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

It is a further object of the present invention to provide for methods of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising a) a replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about 450 (preferably, 1-450) and, preferably, 3511-3523 of a wildtype adenovirus sequence, and, b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or

immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a

It is also an object of the present invention to provide various alternatives for vaccine administration regimes, namely administration of one or more adenoviral and/or DNA plasmid vaccines described herein to provide effective immunoprophylaxis for uninfected individuals or a therapeutic treatment for HIV infected patients. Such processes include but are not limited to multivalent HIV-1 vaccine compositions, various combined modality regimes as well as various prime/boost alternatives. These methods of administration, relating to vaccine composition and/or scheduled administration, will increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

As used throughout the specification and claims, the following definitions and abbreviations are used:

"HAART" refers to -- highly active antiretroviral therapy --.

"first generation" vectors are characterized as being replication-defective.

They typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region as well.

"AEX" refers to Anion Exchange chromatography.

"QPA" refers to Quick PCR-based Potency Assay.

"bps" refers to basepairs.

"s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.

"PBMCs" refers to peripheral blood monocyte cells.

"FL" refers to full length.

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"FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.

"Ad5-Flgag" refers to an adenovirus serotype 5 replication deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.

"Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by activating sequences such as enhancers or inhibiting sequences such as silencers.

"Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results a protein having an N-terminal peptide extension, often referred to as a pro-sequences.

"Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and is therefore not transcribed into mRNA not translated into protein.

"Immunologically relevant" or "biologically active" means (1) with regards to a viral protein, that the protein is capable, upon administration, of eliciting a measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual; or (2) with regards to a nucleotide sequence, that the sequence is capable of encoding for a protein capable of the above.

"Cassette" refers to a nucleic acid sequence which is to be expressed, along with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.

"bGHpA" refers to the bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the leader sequence of the tissue plasminogen activator leader sequence and an optimized HIV gag gene, as exemplified in Figure 30A-B, whether in a DNA or adenovirus-based vaccine vector.

Where utilized, "IA" or "inact" refers to an <u>inactivated</u> version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

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In general, adenoviral constructs, gene constructs are named by reference to the genes contained therein. For example:

"Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal. The transgene was inserted in the E1 antiparallel orientation in an E1 and E3 deleted adenovector.

"MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector taught herein which is deleted of E1, comprises basepairs 1-450 and 3511-3523, and has a human codon-optimized HIV-1 gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct also comprises a bovine growth hormone polyadenylation signal.

"pV1JnsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

"pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

"pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

"pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning

site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdelE1sp1A" or "MRKpdelE1(Pac/pIX/pack450)" or

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"MRKpdelE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) sequences from bp1 to bp450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1 antiparallel) orientation)

"MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intronA) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *BgI*II site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid. This shuttle vector, as shown in Figures 22 and 23, was used to insert the respective IApol and G2A,LLAA nef genes directly into.

"MRKpdelE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from basepairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdelE1 shuttle +hCMV-FL-gag-BGHpA"

"MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-BGHpA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or "pAd5gag2".

"pV1Jns-HIV-pol inact(opt)" or "pV1Jns-HIV IA pol (opt) is the inactivated Pol gene (contained within SEQ ID NO:3) cloned into the BgIII site of V1Jns (Figure 17A-C). As noted herein, various derivatives of HIV-1 pol may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdel+hCMVmin+FL-pol+bGHpA(s)" is the "MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" shuttle mentioned above which contains the IA pol gene is the proper orientation. This shuttle vector is used in a bacterial recombination with MRKpAd(E1-/E3+)Cla1.

"MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+", also referred to herein as "pMRKAd5pol", is the pre-adenovirus plasmid which comprises a CMV-pol inact(opt)-pGHpA construct. The construction of this pre-adenovirus plasmid is shown in Figure 22.

"pV1Jns/nef (G2A,LLAA)" or "V1Jns/opt nef (G2A,LLAA)" comprises codon optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175 (SEQ ID NO:13; which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662). This fragment is subcloned into the Bgl II site of V1Jns and/orV1Jns-tPA (Figures 16A-B). As noted above for HIV-1 pol, HIV-1 nef constructs may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdelE1hCMVminFL-nefBGHpA(s)", also referred to herein as "pMRKAd5nef", is the pre-adenovirus plasmid which comprises a CMV-nef (G2A,LLAA) codon optimized sequence. The construction of this pre-adenovirus plasmid is shown in Figure 23.

#### BRIEF DESCRIPTION OF THE FIGURES

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Figure 1 shows the original HIV-1 gag adenovector (Ad5HIV-1gag). This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

Figure 2 shows the nucleic acid sequence (SEQ ID NO: 29) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the new transgene constructs in comparison with the original gag transgene.

Figure 4 shows the modifications made to the original adenovector backbone in the generation of the novel vectors of the instant invention.

Figure 5 shows the virus mixing experiments that were carried out to determine the effects of the addition made to the packaging signal region (Expt. #1) and the E3 gene on viral growth (Expt. #2). The bars denote the region of modifications made to the E1 deletion.

Figure 6 shows an autoradiograph of viral DNA analysis following the viral mixing experiments described in Examples 6 and 7.

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Figures 7A, 7B and 7C are as follows: Figure 7A shows the hCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7B shows the hCMV-Flgag-SPA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Again, both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7C shows the mCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Once again, both E1 parallel and E1 antiparallel transgene orientation are represented.

Figure 8A shows the experiment designed to test the effect of transgene orientation.

Figure 8B shows the experiments designed to test the effect of polyadenylation signal.

Figure 9 shows viral DNA from the four adenoviral vectors tested (Example 12) at P5, following *Bst*E11 digestion.

Figure 10 shows viral DNA analysis of passages 11 and 12 of MRKpAdHVE3, MRKAd5HIV-1gag, and MRKAd5HIV-1gagE3-.

Figure 11 shows viral DNA analysis (*Hind*III digestion) of passage 6 MRKpAdHVE3 and MRKAd5HIV-1gag used to initiate the viral competition study. The last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI of 280 viral particles).

Figure 12 shows viral DNA analysis by *Hind* III digestion on high passage numbers for MRKAd5HIV-1gag in serum-containing media with collections made at specified times. The first lane shows the 1kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hind*III), MRKAd5HIV-1gag at P16, P19, and P21.

Figure 13 shows serum anti-p24 levels at 3 wks post i.m. immunization of balb/c mice (n=10) with varying doses of several Adgag constructs: (A) MRK Ad5 HIV-1 gag (through passage 5); (B) MRKAd5 hCMV-FLgag-bGHpA (E3-); (C) MRKAd5 hCMV-FLgag-SPA (E3+); (D) MRKAd5 mCMV-FLgag-bGHpA (E3+);

(E) research lot (293 cell-derived) of Ad5HIV-1 gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1 gag. Reported are the geometric mean titers (GMT) for each cohort along with the standard error bars.

Figure 14 shows a restriction map of the pMRKAd5HIV-1gag vector.

Figures 15A-X illustrates the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:27.[coding] and SEQ ID NO:28 [non-coding]).

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Figures 16A-B shows a schematic representation of DNA vaccine expression vectors V1Jns (A) and V1Jns-tPA (B), which are utilized for HIV-1 gag, pol and nef constructs in various DNA/viral vector combined modality regimens as disclosed herein.

Figures 17A-C shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of IA-Pol. Underlined codons and amino acids denote mutations, as listed in Table 1.

Figure 18 shows codon optimized nucleotide and amino acid sequences through the fusion junction of tPA-pol inact(opt) (contained within SEQ ID NOs: 7 and 8, respectively). The underlined portion represents the NH<sub>2</sub>-terminal region of IA-Pol.

Figures 19A-B show a nucleotide sequence comparison between wild type nef(jrfl) and codon optimized nef. The wild type nef gene from the jrfl isolate consists of 648 nucleotides capable of encoding a 216 amino acid polypeptide. WT, wild type sequence (SEQ ID NO:19); opt, codon-optimized sequence (contained within SEQ ID NO:1). The Nef amino acid sequence is shown in one-letter code (SEQ ID NO:2).

Figures 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine174 and 175 are the sites involved in myristylation and dileucine motif, respectively. For both versions of the tpanef fusion genes, the putative leader peptide cleavage sites are

indicated with "\*", and a exogenous serine residue introduced during the construction of the mutants is underlined.

Figure 22 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Pol.

Figure 23 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Nef.

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Figure 24 shows a comparison of clade B vs. clade C anti-gag T cell responses in clade B HIV-infected subjects.

Figure 25 shows a comparison of clade B vs. clade C anti-nef T cell responses in clade B HIV-infected subjects.

Figures 26A-AO illustrates the nucleotide sequence of the pMRKAd5HIV-1pol adenoviral vector (SEQ ID NO:32 [coding] and SEQ ID NO:33 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO3).

Figures 27A-AM illustrates the nucleotide sequence of the pMRKAd5HIV-1 nef adenoviral vector (SEQ ID NO:34 [coding] and SEQ ID NO:35 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO13).

Figure 28 shows the stability of MRKAd5 vectors comprising various promoter fragments (hCMV or mCMV) and terminations signals (bGH or SPA) in E3(+) or E3(-) backbones.

Figures 29A and B shows the anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36, 48, and 60 hpi time points (Figure 29A) and the timcourse QPA supernatant titers (Figure 29B) for MRKAd5gag, MRKAd5pol and MRKAd5nef.

Figure 30 shows the nucleotide sequence (SEQ ID NO:36) and amino acid sequence (SEQ ID NO:37) comprising the open reading frame of a representative tPA-gag fusion for use in the DNA and/or adenoviral vaccine disclosed herein.

Figure 31 shows the intracellular γIFN staining of PBMCs collected at week 10 (post DNA prime) and week 30 (post Ad boost). The cells were stimulated overnight in the presence or absence of the gag peptide pool. They were subsequently stained using fluorescence-tagged anti-CD3, anti-CD8, anti-CD4, and anti-γIFN monoclonal antibodies. Each plot shows all CD3+ T cells which were segregated in terms of positive staining for surface CD8 and γIFN production. The numbers in the upper right and lower right quadrants of each plot are the percentages of CD3<sup>+</sup> cells that were CD8<sup>+</sup>γIFN<sup>+</sup> and CD4<sup>+</sup>γIFN<sup>+</sup>, respectively.

Figure 32 shows a comparison of single-modality adenovirus immunization with DNA + adjuvant prime/adenovirus boost immunization.

Figures 33A-B show the nucleotide sequence (SEQ ID NO: 38) of the open reading frame for the gag-IApol fusion of Example 29.

Figures 34A-B show the protein sequence (SEQ ID NO:39) of the gag-IApol fustion frame.

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#### DETAILED DESCRIPTION OF THE INVENTION

A novel replication-defective, or "first generation," adenoviral vector suitable for use in gene therapy or nucleotide-based vaccine vectors is described. This vector is at least partially deleted in E1 and comprises a wildtype adenovirus cis-acting packaging region from about base pair 1 to between about base pair 342 (more preferably, 400) to about 458 (preferably, 1-450) and, preferably, 3511-3523 of a wild-type adenovirus sequence. It has been found that a vector of this description possesses enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, and is more potent allowing lower doses of virus to be used to generate equivalent immunity. The vector, furthermore, generates a harvested recombinant adenovirus which shows greater cellular-mediated immune responses than replication-deficient vectors not comprising this region (basepairs 342-450). Adenoviral constructs derived from these vectors are, further, very stable genetically, particularly those comprising a transgene under the control of a hCMV promoter devoid of intron A. Viruses in accordance with this description were passaged continually and analyzed; see Example 12. Each virus analyzed maintained it correct genetic structure. Analysis was also carried out under propagation conditions similar to that performed in large scale production. Again, the vectors were found to possess enhanced genetic stability; see Figure 12. Following 21 passages, the viral DNA showed no evidence of rearrangement, and was highly reproducible from one production lot to the next. The outcome of all relevant tests indicate that the adenoviral vector is extremely well suited for large-scale production of recombinant, replication-deficient adenovirus, as shown herein with the data associated with Figure 28.

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A preferred adenoviral vector in accordance with this description is a vector comprising basepairs 1-450, which is deleted in E3. This vector can accommodate up to approximately 7,500 base pairs of foreign DNA inserts (or exogenous genetic material). Another preferred vector is one retaining E3 which comprises basepairs 1-450. A preferred vector of this description is an E3+ vector comprising basepairs 1-450 and 3511-3523. This vector, when deleted of the region spanning basepairs 451-3510, can accommodate up to approximately, 4,850 base pairs of foreign DNA inserts

(or exogenous genetic material). The cloning capacities of the above vectors have been determined using 105% of the wildtype Ad5 sequence as the upper genome size limit.

Wildtype adenovirus serotype 5 is used as the basis for the specific basepair numbers provided throughout the specification. The wildtype adenovirus serotype 5 sequence is known and described in the art; see, Chroboczek et al., 1992 J. Virology 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is a vector based on the adenovirus serotype 5 sequence. One of skill in the art can readily identify the above regions in other adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42), regions defined by basepairs corresponding to the above basepair positions given for adenovirus serotype 5. Accordingly, the instant invention encompasses all adenoviral vectors partially deleted in E1 comprising basepairs corresponding to 1-450 (particularly, 342-450) and, preferably, 3511-3523 of a wild-type adenovirus serotype 5 (Ad5) nucleic acid sequence. Particularly preferred embodiments of the instant invention are those derived from adenoviruses like Ad5 which are classified in subgroup C (e.g., Ad2).

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Vectors in accordance with the instant invention are at least partially deleted in E1. Preferably the E1 region is completely deleted or inactivated. Most preferably, the region deleted of E1 is within basepairs 451-3510. It is to be noted that the extended 5' and 3' regions of the disclosed vectors are believed to effectively reduce the size of the E1 deletion of previous constructs without overlapping any part of the E1A/E1B gene present in the cell line used, i.e., the PER.C6® cell line transefected with base pairs 459-3510. Overlap of adenoviral sequences is avoided because of the possibility of recombination. One of ordinary skill in the art can certainly appreciate that the instant invention can, therefore, be modified if a different cell line transfected with a different segment of adenovirus DNA is utilized. For purposes of exemplification, a 5' region of base pairs 1 to up to 449 is more appropriate if a cell line is transfected with adenoviral sequence from base pairs 450-3510. This holds true as well in the consideration of segments 3' to the E1 deletion.

Preferred embodiments of the instant invention possess an intact E3 region (i.e., an E3 gene capable of encoding a functional E3). Alternate embodiments have a partially deleted E3, an inactivated E3 region, or a sequence completely deleted of E3. Applicants have found, in accordance with the instant invention, that virus comprising the E3 gene were able to amplify more rapidly compared with virus not comprising an E3 gene; see Figure 6 wherein a diagnostic CsCl band corresponding to the E3+ virus

tested (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. These results were obtained following a virus competition study involving mixing equal MOI ratio (1:1) of adenovectors both comprising the E3 gene and not comprising the E3 gene. This increased amplification capacity of the E3+ adenovectors was subsequently confirmed with growth studies; see Table 4A, wherein the E3+ virus exhibit amplification ratios of 470, 420 and 320 as compared with the 115 and 40-50 of the E3- constructs.

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As stated above, vectors in accordance with the instant invention can accommodate up to approximately 4,850 base pairs of exogenous genetic material for an E3+ vector and approximately 7,500 base pairs for an E3- vector. Preferably, the insert brings the adenoviral vector as close as possible to a wild-type genomic size (e.g., for Ad5, 35,935 basepairs). It is well known that adenovirus amplifies best when they are close to their wild-type genomic size.

The genetic material can be inserted in an E1-parallel or an E1 anti-parallel orientation, as such is illustrated in Figure 7A, 7B, 7C and Figure 8A. Particularly preferred embodiments of the instant invention, have the insert in an E1-parallel orientation. Applicants have found, via competition experiments with plasmids containing transgenes in differing orientation (Figure 8A), that vector constructs with the foreign DNA insert in an E1-parallel orientation amplify better and actually outcompete E1-antiparallel-oriented transgenes. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation as compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested.

Adenoviral vectors in accordance with the instant invention are particularly well suited to effectuate expression of desired proteins, one example of which is an HIV protein, particularly an HIV full length gag protein. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription terminator.

The transcriptional promoter is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al, 1991 *Nucl. Acids Res*19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred

for use within the instant adenoviral vector is a human CMV promoter without intronic sequences, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV), constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate (Examples 1-3) comparable expression capabilities *in vitro* when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice *in vivo* with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 µg and 200 µg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

In preferred embodiments, the promoter may also comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought.

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The combination of the CMV promoter (devoid of the intron A region) with the BGH terminator is particularly preferred although other promoter/terminator combinations in the context of FG adenovirus may also be used.

Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA. Examples include but are not limited to the various tPA-gag, tPA-pol and tPA-nef adenovirus-based vaccines disclosed throughout this specification.

In view of the improved adenovirus vectors described herein, an essential portion of the present invention are adenoviral-based HIV vaccines comprising said adenovirus backbones which may be administered to a mammalian host, preferably a human host, in either a prophylactic or therapeutic setting. The HIV vaccines of the present invention, whether administered alone or in combination regimens with other viral- or non-viral-based DNA vaccines, should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load

subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen (e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be utilized in the herein described recombinant adenoviral vectors, preferred embodiments include the codon optimized p55 gag antigen (herein exemplified as MRKAd5gag), pol and nef. Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on concensus Clade B sequences. Preferred versions of the MRKAd5pol and MRKAd5nef series of adenoviral vaccines will encode modified versions of pol or nef, as discussed herein. Preferred embodiments of the MRKAd5HIV-1 vectors carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized env sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24, 1997, respectively; both documents of which are hereby incorporated by reference.

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A most preferred aspect of the instant invention is the disclosed use of the adenoviral vector described above to effectuate expression of HIV gag. Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. As shown in Example 25, a clade B or clade C based p55 gag antigen will potentially be useful on a global scale. As noted herein, the transgene of choice for insertion in to a DNA or MRKAd-based adenoviral vector of the present invention is a codon optimized version of p55 gag. Such a MRKAd5gag adenoviral vector is documented in Example 11 and is at least referred to herein as MRKAd5HIV-1gag. Of course, additional versions are contemplated, including but not limited to modifications such as promoter (e.g., mCMV for hCMV) and/or pA-terminations signal (SPA for bGH) switching, as well as generating MRK Ad5 backbones with or without deletion of the Ad5 E3 gene.

The present invention also relates a series of MRKAd5pol-based adenoviral vaccines which are shown herein to generate cellular immune responses subsequent to administration in mice and non-human primate studies. Several of the MRKAd5pol series are exemplified herein. One such adenoviral vector is referred to as MRKAd5hCMV-inact opt pol(E3+), which comprises the MRKAd5 backbone, the hCMV promoter (no intron A), an inactivated pol transgene, and contains the Ad5 E3 gene in the adenoviral backbone. A second exemplified pre-adenovirus plasmid and concomitant virus is referred to as MRKAd5hCMV-inact opt pol(E3-), which is identical to the former adenoviral vector except that the E3 is deleted. Both constructions contain a codon optimized, inactivated version of HIV-1 Pol, wherein at 10 least the entire coding region is disclosed herein as SEQ ID NO:3 and the expressed protein is shown as SEQ ID NO:4 (see also Figure 17A-C and Table 1, which show targeted deletion for inactivated pol. This and other preferred codon optimized versions of HTV Pol as disclosed herein are essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International 15 Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference. As disclosed in the above-mentioned documents, the open reading frame for these codon-optimized HIV-1 Pol-based DNA vaccines are represented by codon optimized DNA molecules encoding codon optimized HIV-1 Pol (e.g. SEQ ID NO:2), codon optimized HIV-1 Pol fused to an 20 amino terminal localized leader sequence (e.g. SEQ ID NO:6), and especially preferable, and exemplified by the MRKAd5-Pol construct in e.g., Example 19, biologically inactivated pol ("inact opt Pol"; e.g., SEQ ID NO:4) which is devoid of significant PR, RT, RNase or IN activity associated with wild type Pol. In addition, a construct related to SEQ ID NO:4 is contemplated which contains a leader peptide at 25 the amino terminal region of the IA Pol protein. A specific construct is ligated within an appropriate DNA plasmid vector containing regulatory regions operatively linked to the respective HIV-1 Pol coding region, with or without a nucleotide sequence encoding a functional leader peptide. To this end, various HIV-1 Pol constructs disclosed herein relate to open reading frames for cloning to the enhanced first 30 generation Ad vectors of the present invention (such a series of MRKAd5pol adenoviral vaccine vectors), including but not limited to wild type Pol (comprising the DNA molecule encoding WT opt Pol, as set forth in SEQ ID NO:2), tPA-opt WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), inact opt Pol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID 35 NO:4), and tPA-inact opt Pol, (comprising the DNA molecule encoding tPA-inact opt

Pol, as set forth in SEQ ID NO:8). The pol-based versions of enhanced first generation adenovirus vaccines elicit CTL and Th cellular immune responses upon administration to the host, including primates and especially humans. As noted in the above, an effect of the cellular immune-directed vaccines of the present invention should be a lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection.

The present invention further relates to a series of MRKAd5nef-based adenoviral vaccines which, similar to HIV gag and pol antigens, generate cellular immune responses subsequent to administration in mice and non-human primate 10 studies. The MRKAd5nef series are exemplified herein by utilizing the improved MRK adenoviral backbone in combination with modified versions of HIV nef. These exemplified MRKAd5nef vectors are as follows: (1) MRKAd5hCMVnef(G2A,LLAA) (E3+), which comprises the improved MRKAd5 backbone, a human CMV promoter an intact Ad5 E3 gene and a modified nef gene: (2) MRKAd5mCMV-15 nef(G2A,LLAA) (E3+), which is the same as (1) above but substituting a murine CMV promoter for a human CMV promoter; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+), which is the same as (2) except that the nef transgene is tpanef(LLAA). Codon optimized versions of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and 20 PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. Particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein 25 is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH2-terminus of the HIV-1 Nef 30 polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and 35 substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein

described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. MRKAd5nef vectors (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) and (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) contain this transgene. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16. The MRKAd5nef vector "MRKAd5mCMV-tpanef(LLAA) (E3+)" contains this transgene.

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Along with the improved MRKAd5gag adenovirus vaccine vector described 15 herein, generation of a MRKAd5pol and MRKAd5nef adenovirus vector provide for enhanced HIV vaccine capabilities. Namely, the generation of this trio of adenoviral vaccine vectors, all shown to generate effective cellular immune responses subsequent to host administration, provide for the ability to administer these vaccine candidates not only alone, but preferably as part of a divalent (i.e., gag and nef, gag and pol, or 20 pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components). Therefore, a preferred aspect of the present invention are vaccine formulations and associated methods of administration and concomitant generation of host cellular immune responses associated with formulating three separate series of MRKAd5based adenoviral vector vaccines. Of course, this MRKAd5 vaccine series based on distinct HIV antigens promotes expanded opportunities for formulation of a divalent 25 or trivalent vaccine, or possibly administration of separate formulations of one or more monovalent or divalent formulations within a reasonable window of time. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen. An example, but certainly not a limitation, would be separate MRKAd5pol vectors, with 30 one vaccine vector expressing wild type Pol (SEQ ID NO:2) and another MRKAd5pol vector expressing inactivated Pol (SEQ ID NO:6). Another example might be separate MRKAd5nef vectors, with one vaccine vector expressing the tPA/LLAA version of Nef (SEQ ID NO:16) and another MRKAd5nef vector expressing the G2A,LLAA modified version of Nef (SEQ ID NO:14). Therefore, the 35 MRKAd5 adenoviral vectors of the present invention may be used in combination

with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

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The present invention also relates to application of a mono-, dual-, or trimodality administration regime of the MRKAd5gag, pol and nef adenoviral vaccine
series in a prime/boost vaccination schedule. This prime/boost schedule may include
any reasonable combination of the MRKAd5gag, pol and nef adenoviral vaccine
series disclosed herein. In addition, a prime/boost regime may also involve other viral
and/or non-viral DNA vaccines. A preferable addition to an adenoviral vaccine
vector regime includes but is not limited to plasmid DNA vaccines, especially DNA
plasmid vaccines that contain at least one of the codon optimized gag, pol and nef
constructions, as disclosed herein.

Therefore, one aspect of this invention is the administration of the adenoviral vector containing the optimized gag gene in a prime/boost regiment in conjunction with a plasmid DNA encoding gag. To distinguish this plasmid from the adenoviralcontaining shuttle plasmids used in the construction of an adenovirus vector, this plasmid will be referred to as a "vaccine plasmid" or "DNA plasmid vaccine". Preferred vaccine plasmids for use in this administration protocol are disclosed in pending U.S. patent application 09/017,981, filed February 3, 1998 and WO98/34640, published August 13, 1998, both of which are hereby incorporated by reference. Briefly, the preferred vaccine plasmid is designated V1Jns-FLgag, which expresses the same codon-optimized gag gene as the adenoviral vectors of this invention (see Figure 2 for the nucleotide sequence of the exemplified optimized codon version of full length p55 gag). The vaccine plasmid backbone, designated V1Jns contains the CMV immediate-early (IE) promoter and intron A, a bovine growth hormone-derived polyadenylation and transcription termination sequence as the gene expression regulatory elements, and a minimal pUC backbone; see Montgomery et al., 1993, DNA Cell Biol. 12:777-783. The pUC sequence permits high levels of plasmid production in E. coli and has a neomycin resistance gene in place of an ampicillin resistance gene to provide selected growth in the presence of kanamycin. Alternatively, a vaccine plasmid which has the CMV promoter deleted of intron A can be used. Those of skill in the art will recognize that alternative vaccine plasmid

vectors may be easily substituted for these specific constructs, and this invention specifically envisions use of such alternative plasmid DNA vaccine vectors.

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Another aspect of the present invention is a prime/boost regimen which includes a vaccine plasmid which encodes an HIV pol antigen, preferably a codon optimized form of pol and also preferably a vaccine plasmid which comprises a nucleotide sequence which encodes a Pol antigen selected from the group of Pol antigens as shown in SEQ ID NOs: 2, 4, 6 and 8. The variety of potential DNA plasmid vaccines which encode various biologically active forms of HIV-1 Pol, wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized wild type Pol (without Pro activity) and various codon optimized inactivated HIV-1 Pol proteins. The HIV-1 pol open reading disclosed herein are especially preferred for pharmaceutical uses, especially for human administration as delivered via a recombinant adenoviral vaccine, especially an enhanced first generation recombinant adenoviral vaccine as described herein. Several embodiments of this portion of the invention are provided in detail below, namely DNA molecules which comprise a HIV-1 pol open reading frame, whether encoding full length pol or a modification or fusion as described herein, wherein the codon usage has been optimized for expression in a mammal, especially a human. Again, these DNA sequences are positioned appropriately within a recombinant adenoviral vector, such as the exemplified recombinant adenoviral vector described herein, so as to promote expression of the respective HIV-1 Pol gene of interest, and subsequent to administration, elicit a host CTL and Th response. Again, these preferred, but in no way limiting, pol genes are as disclosed herein and essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference.

A third series of vaccine plasmids which are useful in a combined modality and/or prime/boost regimen are vaccine plasmids which encode an HIV nef antigen or biologically and/or immunologically relevant modification thereof. As noted elsewhere, preferred vaccine plasmids contain a codon optimized form of nef and also preferably comprise a nucleotide sequence which encodes a Nef antigen selected from the group of Nef antigens as shown in SEQ ID NOs: 10, 12, 14 and 16. These preferred nef coding regions are disclosed herein, as well as being described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International

Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference.

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Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly is pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

Furthermore and in the alternative, multiple HIV-1 viral antigens, such as the MRKAd5 adenoviral vaccines disclosed herein, may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possible a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in International Publication No. WO 95/24485, which is hereby incorporated by reference. Figure 9 shows that the use of multiple promoters and termination sequences provide for similar growth properties, while Figure 28 shows that these MRKAd5gag-based vectors are also stable at least through passage 21. In the absence of the use of IRES-based technology, it is preferred that a distinct promoter be used to support each respective open reading frame, so as to best preserve vector stability. As examples, and certainly not as limitations, potential multiple transgene vaccines may

include a three transgene vector such as hCMV-gagpol-bGHpA + mCMV-nef-SPA in an E3 deleted backbone or hCMV-gagpol-bGHpA + mCMV-nef-SPA(E3+). Potential "2+1" divalent vaccines of the present invention might be a hCMV-gagbGHpA + mCMV-nef-SPA in an E3+ backbone (vector #1) in combination with hCMV-pol-bGHpA in an E3+ backbone (vector #2), with all transgenes in the E1 parallel orientation. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (e.g.,, nef-pol and gag-nef). These adenoviral compositions are, as above, preferably delivered along with an adenoviral composition comprising an additional HIV antigen in order to diversify the immune response generated upon administration. Therefore, a multivalent vaccine delivered in a single, or possible second, adenoviral vector is certainly contemplated as part of the present invention. Again, this mode of administration is another example of whereby an efficaceous adenovirus-based HIV-1 vaccine may be administered via a combined modality regime. It is important to note, however, that in terms of deciding on an insert for the disclosed adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the adenovirus vehicle. Adenovirus has been shown to exhibit an upper cloning capacity limit of approximately 105% of the wildtype Ad5 sequence.

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Regardless of the gene chosen for expression, it is preferred that the sequence be "optimized" for expression in a human cellular environment. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon. Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood, alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC, CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon frequencies for microorganisms has revealed endogenous DNA of E. coli most commonly contains the CTG leucine-specifying codon, while the DNA of yeasts and slime molds most commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-

rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully transformed host organisms—a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of this invention is an adenovirus vector or adenovirus vector in some combination with a vaccine plasmid where both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HIV gene and, particularly, HIV gag, pol or nef.

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Adenoviral vectors in accordance with the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" *Advances in Pharmacology* 40:137-206, which is hereby incorporated by reference.

In constructing the adenoviral vectors of this invention, it is often convenient to insert them into a plasmid or shuttle vector. These techniques are known and described in Hitt et al., *supra*. This invention specifically includes both the adenovirus and the adenovirus when inserted into a shuttle plasmid.

Preferred shuttle vectors contain an adenoviral portion and a plasmid portion. The adenoviral portion is essentially the same as the adenovirus vector discussed supra, containing adenoviral sequences (with non-functional or deleted E1 and E3 regions) and the gene expression cassette, flanked by convenient restriction sites. The plasmid portion of the shuttle vector often contains an antibiotic resistance marker under transcriptional control of a prokaryotic promoter so that expression of the antibiotic does not occur in eukaryotic cells. Ampicillin resistance genes, neomycin resistance genes and other pharmaceutically acceptable antibiotic resistance markers may be used. To aid in the high level production of the polynucleotide by

fermentation in prokaryotic organisms, it is advantageous for the shuttle vector to contain a prokaryotic origin of replication and be of high copy number. A number of commercially available prokaryotic cloning vectors provide these benefits. It is desirable to remove non-essential DNA sequences. It is also desirable that the vectors not be able to replicate in eukaryotic cells. This minimizes the risk of integration of polynucleotide vaccine sequences into the recipients' genome. Tissue-specific promoters or enhancers may be used whenever it is desirable to limit expression of the polynucleotide to a particular tissue type.

In one embodiment of this invention, the pre-plasmids (e.g., pMRKAd5pol, pMRKAd5nef and pMRKAd5gag were generated by homologous recombination using the MRKHVE3 (and MRKHVO for the E3- version) backbones and the appropriate shuttle vector, as shown for pMRKAd5pol in Figure 22 and for pMRKAd5nef in Figure 23. The plasmid in linear form is capable of replication after entering the PER.C6<sup>®</sup> cells and virus is produced. The infected cells and media were harvested after viral replication was complete.

Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6<sup>®</sup>. Both these cell lines express the adenoviral E1 gene product. PER.C6<sup>®</sup> is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6<sup>®</sup>, from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 J. Gen. Virol 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is important that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

It has been found that vectors generated in accordance with the above description are more effective in inducing an immune response and, thus, constitute very promising vaccine candidates. More particularly, it has been found that first generation adenoviral vectors in accordance with the above description carrying a codon-optimized HIV gag gene, regulated with a strong heterologous promoter can be used as human anti-HIV vaccines, and are capable of inducing immune responses.

Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA immunogens of this invention.

A vaccine composition comprising an adenoviral vector in accordance with the instant invention may contain physiologically acceptable components, such as buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM MgCl<sub>2</sub>; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used it make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM MgCl<sub>2</sub>, 0.005% polysorbate 80 at pH 8.0 This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface. It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

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The amount of adenoviral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene product. In general, an immunologically or prophylactically effective dose of  $1 \times 10^{7}$  to  $1 \times 10^{12}$  particles and preferably about  $1 \times 10^{10}$  to  $1 \times 10^{11}$  particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also contemplated that booster vaccinations are to be provided. Following vaccination with HTV adenoviral vector, boosting with a subsequent HTV adenoviral vector and/or plasmid may be desirable. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine compositions of this invention is also advantageous.

The adenoviral vector and/or vaccine plasmids of this invention polynucleotide may be unassociated with any proteins, adjuvants or other agents which impact on the recipients' immune system. In this case, it is desirable for the vector to be in a physiologically acceptable solution, such as, but not limited to, sterile saline or sterile buffered saline. Alternatively, the vector may be associated with an adjuvant known in the art to boost immune responses (i.e., a "biologically effective"

adjuvant), such as a protein or other carrier. Vaccine plasmids of this invention may, for instance, be delivered in saline (e.g., PBS) with or without an adjuvant. Preferred adjuvants are Alum or CRL1005 Block Copolymer. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used to advantage. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention.

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This invention also includes a prime and boost regimen wherein a first adenoviral vector is administered, then a booster dose is given. The booster dose may be repeated at selected time intervals. Alternatively, a preferred inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype. More preferably, the inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype, wherein the first and second adenovirus serotypes are classified within separate subgroups of adenoviruses. The above prime/boost schemes are particularly preferred in those situations where a preexisting immunity is identified to the adenoviral vector of choice. In this type of scheme, the individual or population of individuals is primed with an adenovirus of a serotype other than that to which the preexisting immunity is identified. This enables the first adenovirus to effectuate sufficient expression of the transgene while evading existing immunity to the second adenovirus (the boosting adenovirus) and, further, allows for the subsequent delivery of the transgene via the boosting adenovirus to be more effective. Adenovirus serotype 5 is one example of a virus to which such a scheme might be desirable. In accordance with this invention, therefore, one might decide to prime with a non-group C adenovirus (e.g., Ad12, a group A adenovirus, Ad24, a group D adenovirus, or Ad35, a group B adenovirus) to evade anti-Ad5 immunity and then boost with Ad5, a group C adenovirus. Another preferred embodiment involves administration of a different adenovirus (including non-human adenovirus) vaccine followed by administration of the adenoviral vaccines disclosed. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

A large body of human and animal data supports the importance of cellular immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate

with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV by remain uninfected; CTL has been noted in several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8 monoclonal antibody abrogated this control of infection and leads to disease progression. This invention uses adenoviral vaccines alone or in combination with plasmid vaccines to induce CTL.

The following non-limiting Examples are presented to better illustrate the invention.

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#### **EXAMPLE 1**

Removal of the Intron A Portion of the hCMV Promoter GMP grade pVIInsHIVgag was used as the starting material to amplify the hCMV promoter. PVIInsHIVgag is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; see Montgomery et al., supra for a description of the plasmid backbone. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the Msc1 site of the hCMV promoter and a 3' primer (designed to contain the BgIII recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity Taq polymerase) which encompassed the entire hCMV promoter (minus intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with Msc1 and BgIII. This fragment was then cloned back into the original GMP grade pV1JnsHIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following Msc1 and BgIII digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA expression cassette within the original pV1JnsHIVgag vector backbone. This vector is designated pVIJnsCMV(no intron).

The FLgag gene was excised from pV1JnsHIVgag using BgIII digestion and the 1,526 bp gene was gel purified and cloned into pV1JnsCMV(no intron) at the BgIII site. Colonies were screened using Sma1 restriction enzymes to identify clones that carried the Flgag gene in the correct orientation. This plasmid, designated pV1JnsCMV(no intron)-FLgag-bGHpA, was fully sequenced to confirm sequence integrity.

Two additional transgenes were also constructed. The plasmid, pV1JnsCMV(no intron)-FLgag-SPA, is identical to pV1JnsCMV(no intron)-FLgag-bGHpA except that the bovine growth hormone polyadenylation signal has been replaced with a short synthetic polyA signal (SPA) of 50 nucleotides in length. The sequence of the SPA is as shown, with the essential components (poly(A) site, (GT)<sub>n</sub>, and (T)<sub>n</sub>; respectively) underlined:

<u>AATAAA</u>AGATCTTTATTTTCATTAGATCT<u>GTGTG TTGGTTTTTTGTGTG</u> (SEQ ID NO:18).

The plasmid, pV1Jns-mCMV-FLgag-bGHpA, is identical to the pV1JnsCMV(no intron)-FLgag-bGHpA except that the hCMV promoter has been removed and replaced with the murine CMV (mCMV) promoter.

Figure 3 diagrammatically shows the new transgene constructs in comparison with the original transgene.

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#### EXAMPLE 2

Gag Expression Assay for Modified Gag Transgenes

Gag Elisa was performed on culture supernatants obtained from transient tissue culture transfection experiments in which the two new hCMV-containing plasmid constructs, pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA, both devoid of intron A, were compared to pV1JnsHIVgag which, as noted above possesses the intron A as part of the hCMV promoter. Table 2 below shows the *in vitro* gag expression data of the new gag plasmids compared with the GMP grade original plasmid. The results displayed in Table 2 show that both of the new hCMV gag plasmid constructs have expression capacities comparable to the original plasmid construct which contains the intron A portion of the hCMV promoter.

Table 2: In vitro DNA transfection of original and new plasmid HIV-1 gag constructs.

Plasmid	μg gag/10e6 COS cells/5μg DNA/48 hr
HIVFL-gagPR9901 <sup>a</sup>	10.8
PVIIns-hCMV-FLgag-bGHpAb	16.6
pV1Jns-hCMV-FLgag-SPA <sup>b,c</sup>	12.0

<sup>&</sup>lt;sup>a</sup> GMP grade pV1Jns-hCMVintronA-FLgag-bGHpA.

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#### **EXAMPLE 3**

Rodent (Balb/c) Study for Modified gag Transgenes
A rodent study was performed on the two new plasmid constructs
described above – pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no
intron)-FLgag-SPA - in order to compare them with the construct described above
possessing the intron A portion of the CMV promoter, pV1JnsHIVgag. Gag antibody
and Elispot responses (described in PCT International Application No.
PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S.
Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S.
Application Serial No. 60/148,981, filed August 13, 1999, all three applications which
are hereby incorporated by reference) were measured. The results displayed in Table
3 below, show that the new plasmid constructs behaved equivalently to the original
construct in Balb/c mice with respect to their antibody and T-cell responses at both
dosages of plasmid DNA tested, 20 μg and 200 μg.

b New plasmid constructions that have the intron A portion removed from the hCMV promoter.

<sup>&</sup>lt;sup>c</sup> In this construct the bGH terminator has been replaced with the short synthetic polyadenylation signal (SPA)

**EXAMPLE 4** 

Table 3: HIV191: Immunogenicity of V1Jns-gag under different promoter and termination control elements.

DNA	Dose, ug <sup>b</sup>	Anti-p24 Titers (3 Wk PD1)°				SFC/10^6 Cells (4 Wk PD1) <sup>d</sup>			
Promoter/terminator		GMT	+SE	-SE	Media	gag197-205	p24		
HIVFL-gagPR9901	200	12800	4652	3412	2(2)	129(19)	30(11)		
(GMP grade)	20	5572	1574	1227	0	56(9)	25(6)		
pV1Jns-hCMV-	200	11143	2831	2257	0	98(5)	12(6)		
FL-gag-bGHpA	20	7352	2808	2032	0	73(9)	11(6)		
pV1Jns-hCMV-	200	16890	5815	4326	1(1)	94(4)	26(7)		
FL-gag-SPA	20	5971	5361	2825	0	85(17)	38(10)		
Naīve	0	123	50	36	0	0	ó		

ain PBS

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#### Construction of the Modified Shuttle Vector - "MRKpdelE1 Shuttle"

The modifications to the original Ad5 shuttle vector (pdelE1sp1A; a vector comprising Ad5 sequences from basepairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:

- (1) The left ITR region was extended to include the *Pac1* site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.
- 10 (2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.
  - (3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).

These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6® cell line. All manipulations were performed by modifying the Ad shuttle vector pdelE1sp1A.

Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbones (pAdHVO and pAdHVE3) by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.

bi.m. Injections into both quads, 50 µL per quad

cn=10;GMT, geometric mean titer; SE, standard. error

dn=5, pooled spleens; mean of triplicate wells and standard, deviation, in parentheses;

PCT/US01/28861 WO 02/22080

#### EXAMPLE 5

#### Construction of Modified Adenovector Backbones (E3+ and E3-)

The original adenovectors pAdHVO (comprising all Ad5 sequences except those nucleotides encompassing the E1 and E3 regions) and pADHVE3 (comprising all Ad5 sequences except those nucleotides encompassing the E1 region), were each 5 reconstructed so that they contained the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdelE1 shuttle) with Pac1 and BstZ1101 and isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from either Cla1 10 linearized pAdHVO (E3- adenovector) or Cla1 linearized pAdHVE3 (E3+adenovector) into E. coli BJ5183 competent cells. At least two colonies from each transformation were selected and grown in Terrific™ broth for 6-8 hours until turbidity was reached. DNA was extracted from each cell pellet and then transformed into E. coli XL1 competent cells. One colony from each transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction 15 digestions to identify correct clones. The modified adenovectors were designated MRKpAdHVO (E3- plasmid) and MRKpAdHVE3 (E3+ plasmid). Virus from these new adenovectors (MRKHVO and MRKHVE3, respectively) as well as the old version of the adenovectors were generated in the PER.C6® cell lines to accommodate the following series of viral competition experiments. In addition, the multiple cloning site of the original shuttle vector contained ClaI, BamHI, Xho I, EcoRV, HindIII, Sal I, and Bgl II sites. This MCS was replaced with a new MCS containing Not I, Cla I, EcoRV and Asc I sites. This new MCS has been transferred to the MRKpAdHVO and MRKpAdHVE3 pre-plasmids along with the modification made to the packaging region and pIX gene.

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#### **EXAMPLE 6**

#### Analysis of the Effect of the Packaging Signal Extension

To study the effects of the modifications made to the E1 deletion region, the viruses obtained from the original backbone (pAdHVE3) and the new backbone 30 (MRKpAdHVE3) were mixed together in equal MOI ratios (1:1 and 5:5) and passaged through several rounds; see Figure 5, Expt.#1. Both of the viruses in the experiment contained the E3 gene intact and did not contain a transgene. The only difference between the two viruses was within the region of the E1 deletion. Following the coinfection of the viruses at P1 (passage 1), the mixtures were 35

propagated through an additional 4 passages at which time the cells were harvested

and the virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids (pAdHVE3 ("OLD E3+"); MRKpAdHVE3 ("NEW E3+")) were also digested with *Hind*III (and *Pac1* to remove the vector backbone) and subsequently labeled with [<sup>33</sup>P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 clearly shows that the new adenovirus which has the addition made to the packaging signal region has a growth advantage compared with the original adenovirus. In the experiments performed (at either ratio tested), only the digestion bands pertaining to the newly modified virus were present. The diagnostic band of size 3,206 (from the new virus) was clearly present. However, there was no evidence of the diagnostic band of size 2,737 bp expected from the original virus.

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#### **EXAMPLE 7**

#### Analysis of the Effect of the E3 Gene

The second set of the virus competition study involved mixing equal MOI ratio (1:1) of the newly modified viruses, that obtained from MRKpAdHVO and MRKpAdHVE3 (Figure 5, Expt. #2). In this set, both viruses had the new modifications made to the E1 deletion. The first virus (that from MRKpAdHVO) does not contain an E3 gene. The second virus (that from MRKpAdHVE3) does contain the E3 gene. Neither of the viruses contain a transgene. Following coinfection of the viruses, the mixtures were propagated through an additional 4 passages at which time the cells were harvested and the total virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with HindIII and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids MRKpAdHVO ("NEW E3-"); MRKpAdHVE3 ("NEW E3+") were also digested with HindIII (and Pac1 to remove the vector backbone) and then labeled with [33P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 shows the results of the viral DNA analysis of the E3+ virus and E3- virus mixing experiment. The diagnostic band corresponding to the E3+ virus (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. This indicates that the virus that contains the E3 gene is able to amplify more rapidly

compared with the virus that does not contain an E3 gene. This increased amplification capacity has been confirmed by growth studies; see Table 4 below.

#### **EXAMPLE 8**

# Construction of the new shuttle vector containing modified gag transgene – "MRKpdelE1-CMV(no intron)-FLgag-bGHpA"

The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHpA was digested with *Msc*1 overnight and then digested with *Sfi*1 for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 mins at 30°C. The DNA mixture was desalted using the Qiaex II kit and then Klenow treated for 30 mins at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdelE1 shuttle) was linearized by digestion with EcoRV, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel and E1 anti-parallel orientation. This strategy was followed to clone in the other gag transgenes in the MRKpdelE1 shuttle vector.

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#### **EXAMPLE 9**

## Construction of the MRK FG Adenovectors

The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdelE1-CMV(no intron)-FLgag-bGHpA, was digested with Pac1. The reaction mixture was digested with BsfZ171. The 5,291 bp fragment was purified by gel extraction. The MRKpAdHVE3 plasmid was digested with Cla1 overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and ~100 ng of linearized MRKpAdHVE3 DNA were co-transformed into E. coli BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml Terrific<sup>TM</sup> broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 μl dH<sub>2</sub>0. A 2 μl aliquot of this DNA was transformed into E. coli XL-1 competent cells. A single colony from each separate transformation was selected and grown overnight in 3 ml LB +100 μg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive clone was identified by digestion with the restriction enzyme BstEII which cleaves

within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size. This strategy was followed to generate E3- and E3+ versions of each of the other gag transgene constructions in both E1 parallel and E1 anti-parallel versions. Figures 7A, 7B and 7C show the various combinations of adenovectors constructed.

#### **EXAMPLE 10**

### Plasmid Competition Studies

A series of plasmid competition studies was carried out. Briefly, the screening of the various combinations of new constructs was performed by mixing equal amounts of each of two competing plasmids. In the experiment shown in Figure 8A, plasmids containing the same transgene but in different orientations were mixed together to create a "competition" between the two plasmids. The aim was to look at the effects of transgene orientation. In the experiment shown in Figure 8B, plasmids containing different polyadenylation signals (but in the same orientation) were mixed together in equal amounts. The aim was to assess effects of polyA signals. Following the initial transfection, the virus was passaged through ten rounds and the viral DNA analyzed by radioactive restriction analysis.

Analysis of the viral species from the plasmid mixing experiment (Figure 8A) showed that adenovectors which had the transgene inserted in the E1 parallel orientation amplified better and were able to out-compete the adenovirus which had the transgene inserted in the E1 anti-parallel orientation. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested (hCMV(no intron)-FLgag-bGHpA and hCMV(no intron)-FLgag-SPA).

Analysis of the viral species from the plasmid mixing experiment #2 (Figure 8B) at passages 3 and 6 showed that the polyadenylation signals tested (bGHpA and SPA) did not have an effect on the growth of the virus. Even at passage 10 the two viral species in the mixture were still present in equal amounts.

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#### **EXAMPLE 11**

Virus generation of an enhanced adenoviral construct - "MRK Ad5 HIV-1gag"

The results obtained from the competition study allowed us to make the following conclusions: (1) The packaging signal extension is beneficial; (2) Presence of E3 does enhance viral growth; (3) E1 parallel orientation is recommended; and (4) PolyA signals have no effect on the growth of the adenovirus.

MRK Ad5 HTV-1 gag exhibited the most desirable results. This construct contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have designated this adenovector MRK Ad5 HTV-1 gag. This construct was prepared as outlined below:

The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested was Pac1 to release the vector backbone and 3.3 µg was transfected by calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing PER.C6® cells at ~60% confluence. Once CPE was reached (7-10 days), the culture was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was used to infect into a 6 cm dish containing PER.C6® cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6<sup>®</sup> cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral DNA was then digested with *Hind*III and radioactively labeled with [33P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with Pac1/HindIII prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued. This strategy was used to rescue virus from each of the various adenovector plasmid constructs prepared.

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#### **EXAMPLE 12**

#### Stability Analyses

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To determine whether the various adenovector constructs (e.g., MRK Ad5 HIV-1 gag) show genetic stability, the viruses were each passaged continually. The viral DNA was analyzed at passages 3, 6 and 10. Each virus maintained its correct genetic structure. In addition, the stability of the MRK Ad5 HIV-1 gag was analyzed under propagation conditions similar to that performed in large scale production. For this analysis, the transfections of MRK Ad5 HIV-1 gag as well as three other adenoviral vectors were repeated and the virus was purified at P3. The three other adenovectors were as follows: (1) that comprising hCMV(no intron)-Flgag with a bGHpA terminator in an E3- adenovector backbone; (2) that comprising hCMV(no intron)-Flgag with a SPA termination signal in an E3+ adenovector backbone, and that comprising a mCMV-Flgag with a bGHpA terminator in an E3+ adenovector backbone. All of the vectors have the transgene inserted in the E1 parallel orientation. Viral DNA was analyzed by radioactive restriction analysis to confirm that it was correct before being delivered to fermentation cell culture for continued passaging in serum-free media. At P5 each of the four viruses were purified and the viral DNA extracted for analysis by the restriction digestion and radiolabeling procedure. This virus has subsequently been used in a series of studies (in vitro gag expression in COS cells, rodent study and rhesus monkey study) as will be described below. The viruses from P5 are shown in Figure 9.

The passaging under serum-free conditions was continued for the MRKHVE3 (transgene-less, obtained from MRKpAdHVE3 pre-plasmid) and the MRKAd5HIV-1gag (obtained from MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA pre-plasmid) viruses. Figure 10 shows viral DNA analysis by radioactive restriction digestion at passage 11 for MRKHVE3, MRKAd5HIV-1gagE3-, and passage 11 and 12 for MRKAd5HIV-1gag. Aside from the first lane which is the DNA marker lane, the next three lanes are virus from the pre-plasmid controls (controls based on the original virus) - MRKpAdHVE3 (also referred to as "pMRKHVE3"), MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA, and pMRKAd5gag(E3-), respectively. As seen in Figure 10, each of the viral DNA samples show the expected bands with no extraneous bands showing. This signifies that there are no major variant adenovirus species present that can be detected by autoradiography.

Figure 11 shows the results of viral competition study between MRKHVE3 and MRKAd5HIV-1gag. These viruses were mixed together at equal MOI (140 viral

particles each; 280 vp total) at passage 6 and continued to be passaged until P11. Aside from the first lane which is the DNA marker lane, the next two lanes are the pre-plasmid controls obtained from MRKpAdHVE3 and MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA. The next two lanes are the viral DNA from the starting viral material at passage six. The last two lanes are the competition studies performed in duplicate. The data in Figure 11 shows the effect the gag transgene in culture. Growth of a MRKAd5gag virus was compared with growth of a "transgene-less" MRKHVE3. These two viruses were infected at the same MOI (i.e. 140 vp each) at passage 6 and then passaged through to passage 11 and the viral pool was analyzed by radioactive restriction analysis. The data shows that one virus did not out compete the other. Therefore, the gag transgene did not show obvious signs of toxicity to the adenovirus.

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Analysis by *Hind*III digestion shows that each virus specie is present in approximately equal amounts. As above, there does not appear to be signs of any extraneous bands. Figure 12 shows higher passage numbers for MRKAd5HIV-1gag grown under serum-containing conditions. The genome integrity again has been maintained and there is no evidence of rearrangements, even at the highest passage level (P21).

Each of the four vectors shown in Figure 9 were analyzed for amplification capacity. Table 4 below shows the QPA analysis used in the estimation of viral amplification ratios at P4. The determination of the amplification ratio for the original HIV-1 gag construct is based on the clinical lot at P12. It has been shown that amplification rates increases with higher passage number for the original virus. The reason for this observation is due to the emergence of variants which exhibit increased growth rates compared to the intact adenovector. With continued passaging of the original Ad gag vector, the level of variants increases and hence amplification rates increase also.

The MRK Ad5 HIV-1 gag virus has also been continually passaged under process conditions (i.e., serum-free media). Viral DNA extracted from passages 11 and 12 show no evidence of rearrangement.

Table 4:
Amplification Ratios Based on AEX and QPA Analysis of Virus Amplification from Passage 3 to Passage 4.

Ad gag construct	Amplification Ratio
MRKAd5gag	470
HCMV-Flgag-bGHpA [E3-]	115
HCMV-Figag-SPA [E3+]	320
mCMV-FLgag-bGHpA [E3+]	420
Original construct *	40 - 50

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#### EXAMPLE 13

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Analytical Evaluation of the enhanced Ad5 Constructs

To study the effects of the transgene and the E3 gene on virus amplification, the enhanced adenoviral vector, MRK Ad5 HIV-1 gag, along with its transgene-less version (MRKpAdHVE3) and its E3- version (MRK Ad5 HIV-1 gag E3-), was studied for several passages under serum-free conditions. Table 5A shows the amplification ratios determined for passages P3 to P8 for MRK Ad5 HIV-1 gag. Within a certain MOI range, it has been determined that the virus output is directly proportional to the virus input. Therefore, the greater the number of virus particles per cell at infection, the greater the virus amount produced. Viral amplification ratios, on the other hand, are inversely proportional to the virus input. The lower the virus input, the greater the amplification ratio.

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Table 5B shows the amplification rates of the new E3+ vector backbone MRKpAdHVE3. It has a significantly lower rate of amplification compared with the gag transgene containing version. This may be contributed to the larger size MRK Ad5 HIV-1 gag since it contains the transgene. This inclusion of the transgene brings the size of the adenovirus closer to the size of a wild type Ad5 virus. It is well known that adenoviruses amplify best when they are at close to their wild type genomic size.

<sup>\*</sup> This estimation is based on the clinical lot growth characteristics at Passage 12.

Wild type Ad5 is 35,935 bp. The MRKpAdHVE3 is 32, 905 bp in length. The enhanced adenovector MRK Ad5 HIV-1 gag is 35,453bp (See Figure 14 for vector map; see also Figure 15A-X show the complete pre-adenoviral vector sequence, which includes an additional 2,021 bp of the vector backbone).

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Table 5C shows the amplification rates of the new E3- gag containing virus MRK Ad5 HIV-1 gag E3-. Once again, this virus shows lower growth rate than the enhanced adenoviral vector. This may be attributed to the decreased sized of this virus (due to the E3 gene deletion) compared with wild type Ad5. The MRK Ad5 HIV-1 gag E3- virus is 32,810 bp in length. This can be compared with the wild type Ad5 which is 35,935 bp and MRK Ad5 HIV-1 gag which is 35,453 bp in length.

**Table 5A:** Amplification ratios determined by AEX and QPA for MRKAd5gag over several continuous passaging in serum free media. Following P5, two replicate samples were taken (rep-1 and rep-2) and analyzed.

# MRKAd5gag rep1

	Xv (10° ce0s/n	ni), Viability (%)	Harvest Time	Cell Passage	Titer	TRes	QPA	Patio	Amplification	AEX
	Infection	Harvest	hpl	Number	10 <sup>so</sup> vp/ml culture	10° vp/ceB	10° TCID <sub>20</sub> /ml	AEX:QPA	Ratto	Internal Control
P4	1.49, 81%	0.58, 50%	44	46	8.7	5.9	1.72	50	470 (MOI = 125)	
P5	1.38, 93%	0.68, 47%	48	49	6.7	4.9	1.38	49	170	
P6	1.04, 94%	0.68, 77%	47	48	5.8	5.6	1.42	41	200	
P7	1.50, 84%	0.96, 61%	49.5	50	3.9	1.4	0.97	40	50	
₽7	1.09, 97%	0.78, 59%	50	52	5.2	4.7	1.70	31	170	
P8	1.03, 94%	0.86, 64%	47.5	54	9.0	8.7	1.10	52	310	
P9	0.89, 95%	0.99, 73%	47.5	56	4,4	4.9	1.03	43	175	3.12 2.84
P10	1.09, 91%	1.06, 66%	47.5	58	3.0	2.8	1.16	26	100	2.70 2.60
P11	1.19, 88%	0.98, 65%	47	60	3.6	3.0	1.15	31	110	2.70 2.70
P12	0.98, 91%	0.85, 63%	47.5	47	5.4	5.5	1.20	45	200	2,86 2,60
P13	1.00, 88%	0.70, 67%	49	49	5.8	5.8	1.11	52	210	3.18 3.18
P14	1.94, 92%	0.88, 67%	46	63	8.6	4.4			160	3,28 3,27
P15	0.97, 96%	0.64, 66%	47	47	6.9	7.1			250	3.12 2.91

**Table 5B:** Amplification ratios determined by AEX and QPA for **MRKHVE3** over several continuous passaging in serum free media. **MRKHVE3** is the new vector backbone which does NOT carry a transgene.

# MRKHVE3

	Xv (10° cells/n	nl), Vlability (%)	Harvest Time	Cell Passage	Titer	Titer	QPA	Ratio	Amplification	AEX
	Infection	Harvest	hpl	Number	10 <sup>10</sup> vp/ml culture	10° vp/cet	10° TCID <sub>sc</sub> /ml	AEX:QPA	Ratio	Internal Control
P4	1.10, 97%	1.28, 79%	49	54	4.1	3.8	1.70	25	300 (MO) = 125)	
P5	0.92, 89%	1.18, 77%	47	. 48	4.3	4.7	1.24	35	170	
P6	1.55, 86%	1.26, 76%	49.5	50	1,2	0.8	0.56	21	30	
P6	1.09, 97%	1.11, 81%	49	52	4.0	3.6	1.16	34	130	
P7	1.17, 91%	1.22, 91%	47.5	54	3.7	3.2	0.50	74	110	
P8	0.98, 88%	1.41, 83%	48	58	2.1	2.1	0.47	45	75	3.12 2.84
P9	1.20, 89%	1.26, 61%	47.5	58	0.8	0.7	0.29	28	25	2.70 2,60
P10	0.99, 82%	1.55, 86%	47	60	2.3	23	0.43	53	80	2.70 2.70
P11	1.07, 98%	1.25, 83%	48	47	2.7	2.5	0.41	66	90	2.86 2.60
P12	0.80, 91%	1,14, 80%	49.5	49	5.9	7.4	0.48	123	260	3.18 3.18
P13	1.96, 95%	1.14, 85%	45.5	53	5.8	3.0			110	3.28 3.27
P14	0.97, 96%	1.03, 98%	48.5	47	9.4	9.7			350	3.12 2.91
P15	0.87, 99%	0.97, 59%	49.5	49	5.3	6.1			218	2.78 2.52

Table 5C. Amplification ratios determined by AEX and QPA for MRKAd5gag(E3-) over several continuous passaging in serum free media. This construct is identical to the MRKAd5gag construct except that this version is DELETED of the E3 gene.

# MRKAd5gag(E3-)

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	Xv (10° pells/n	ni), Vlability (%)	Harvest Time	Cell Passage	Titer	Titer	QPA	Ratio	Amplification	AEX
	Infection	Harvest	h.p.l.	Number	10 <sup>19</sup> vp/ml culture	10 <sup>4</sup> vp/ceil	10° TCID <sub>co</sub> /ml	AEX;QPA	Ratio	Internal Control
P4	1.62, 77%	1.12, 62%	47.5	46	2,0	1.2	0.92	20	100 (MOI=125)	
P5	1.16, 92%	0.62, 43%	49	49	3.3	2.9	0.99	34	100	
P6	1.71, 86%	0.20, 10%	49	50	4.7	2.7	1.70	28	100	1
P6	1.09, 97%	0.63, 54%	49.5	52	5.4	5.0	1.76	31	180	
P7	1.17, 91%	0.98, 72%	47.50	54	7.1	6.1	0.67	106	220	
P8	0.98, 88%	0.77, 48%	48	56	3.1	3.2	0.66	47	115	3.12 2.84
P9	1.20, 89%	1.03, 72%	48	58	1.8	1.5	0.57	32	55	2.70 2.60
P10	0.99, 82%	0.80, 62%	46.5	60	3,2	3.2	0.68	47	115	2.70 2.70
P11	1.07, 95%	0.98, 70%	48.5	47	5.9	5.5	0.68	87	200	2.86 2.60
P12	0.80, 91%	0.67, 59%	50	49	5.1	6.4	0.72	71	230	3.18 3.18
P13	1.96, 95%	0.91, 59%	45.5	53	7.4	3.5			135	3.28 3.27
P14	0.97, 96%	0.81, 74%	48	47	6.8	7.0			250	3.12 2.91
P15	0.87, 99%	0.84, 56%	49	49	4.8	5.5			196	2.78 2.52

#### **EXAMPLE 14**

#### Gag Expression Analysis of the Novel Constructs

In vitro gag analysis of the MRK Ad5 HIV-1 gag and the original HIV-gag vectors (research and clinical lot) show comparable gag expression. The clinical lot shows only a slightly reduced gag expression level. The most noticeable difference is with the mCMV vector. This vector shows roughly 3 fold lower expression levels compared with the other vectors tested (which all contain hCMV promoters). The mCMV-FLgag with bGHpA assay was performed three times using different propagation and purification lots and it consistently exhibited weaker gag expression.

#### **EXAMPLE 15**

# Evaluation of MRK Ad5 HIV-1 gag and Other gag-Containing Adenovectors in Balb/c Mice

Cohorts of 10 balb/c mice were vaccinated intramuscularly with escalating doses of MRK Ad5 HIV-1 gag, and the research and clinical lots of original Ad5HIV-1gag. Serum samples were collected 3 weeks post dose 1 and analyzed by anti-p24 sandwich ELISA.

Anti-p24 titers in mice that received MRK Ad5 HIV-1 gag (107 and 109 vp(viral particle) doses) were comparable (Figure 13) to those of the research lot of Ad5HIV-1 gag, for which much of the early rhesus data were generated on. These titers were also comparable when E3 is deleted (MRKAd5hCMVgagbGHpA(E3-)) or SPA is substituted for bGHpA terminator (MRKAd5 hCMV-gag-SPA (E3+)) or murine CMV promoter is used in place of hCMV (MRKAd5 mCMV-gag-bGHpA (E3+)) in the MRKAd5 backbone.

The results shown in Table 7 indicate that the three other vectors (in addition to the preferred vector, MRK Ad5 HIV-1 gag, are also capable of inducing strong anti-gag antibody responses in mice. Interestingly enough, while the mCMV-FLgag construct containing bGHpA and E3+ in an E1 parallel orientation showed lowest gag expression in the COS cell *in vitro* infection (Table 6) in comparison with the other vectors tested, it generated the greatest anti-gag antibody response this *in vivo* Balb/c study. Table 7 also shows a dose response in anti-gag antibody production in both the research and the clinical lot. As expected, the clinical lot shows reduced anti-gag antibody induction at each dosage level compared to the same dosage used for the research lot.

Table 6: In vitro analysis for gag expression in COS cells by Elisa assay.

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Viral Vectors <sup>a</sup>	μg gag/4.8x10e5 COS/10e8 parts/48hr
MRKAd5gag <sup>b</sup>	1.40
Clinical lot Ad5gag <sup>c</sup>	1.28
Research lot Ad5gag <sup>d</sup>	1.32
MCMVFL-gagbGHpA <sup>e</sup>	0.42

<sup>&</sup>lt;sup>a</sup> A<sub>260nm</sub> absorbance readings taken for viral particle determinations.

<sup>&</sup>lt;sup>b</sup> MRKAd5gag was produced in serum free conditions and purified at P5.

<sup>&</sup>lt;sup>c</sup> Clinical lot# Ad5gagFN0001

<sup>25</sup> d Research Ad5FLgag lot# 6399

e mCMVFL-gagbGHpA was produced in serum free conditions and purified at P5.

Table 7: mHIV020 Anti-p24 Ab Titers in Balb/c mice (n=10) vaccinated with various Adgag constructs and lots (3 week post dose1).

Group	Vaccine	Dose	GMT	SE upper	SE lower
ID		(vp)	ļ		
1	<sup>a</sup> MRKAd5gag	10^7 10^9	25600 409600	5877 94028	4780 76473
2	, "	10.9	409000	94020	70473
3	hCMV FL-gag bGHpA [E3-] →	10^7	7352	2077	1620
4	"	10^9	235253	59767	47659
5	hCMV FL-gag SPA [E3+] →	10^7	12800	9905	236
6		10^9	310419	99181	75165
7	<sup>b</sup> mCMV FL-gag bGHpA [E3+] →	10^7	44572	23504	15389
8	" " " " " " " " " " " " " " " " " " "	10^9	941014	239068	190636
9	°hCMV FL-gag bGHpA <b>[E3-]</b> ←	10^7	3676	934	745
10		10^9	117627	17491	15227
11	research lot hCMV intronA FL-gag bGHpA [E3-] <-	10^6	528	262	175
12	и	10^7	14703	5274	3882
13	n i	10^8	58813	14942	11915
14	. "	10^9	204800	53232	42250
15	clinical lot hCMVintronA FL-gag bGHpA [E3-] <-	10^6	230	82	61
16	Ginneal for Homanitative F-868 posible [Fo ]	10^7	4222	3405	1138
17		10^8	19401	3939	3274
18	· 13	10^9	89144	25187	19639
19	Naïve	none	93	7	6

\*2x50 µL i.m. (quad) injections/animal

P.I.s: Youil, Chen, Casimiro Vaccination: T. Toner, Q. Su

Assay: M. Chen

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<sup>a</sup>The structure of MRKAd5gag is: hCMVFL-gagbGHpA [E3+] → The <u>same lot</u> of MRKAd5gag used in this rodent study was used in the Rhesus monkey study (Tables 7 and 8).

<sup>b</sup>The same lot of mCMVFL-gagbGHpA[E3+] used in the *in vitro* study (Table 6) ws used here.

<sup>c</sup>This construct was designed by Volker Sandig. It contains a shorter version of the hCMV promoter than that used in the MRK constructs. The adenovector backbone is identical to the original backbone used in the original Adgag vector. Expression at 10e7 dose from this vector is 7 fold lower than the same dose of the MRKAd5gag and 4 fold lower than the research lot.

#### **EXAMPLE 16**

Comparison of Humoral and Cellular Responses Towards the Original Ad-gag Construct with the New MRK Ad5 HIV-1 gag in Rhesus Monkeys

Cohorts of 3 rhesus monkeys were vaccinated intramuscularly with MRK Ad5 HIV-1 gag or the clinical Ad5gag bulk at two doses,  $10^{11}$  vp and  $10^{9}$  vp. Immunizations were conducted at week 0, 4, and 25. Serum and PBMC samples were collected at selected time points. The serum sample were assayed for anti-p24 Ab titers (using competitive based assay) and the PBMCs for antigen-specific IFN-gamma secretion following overnight stimulation with gag 20-mer peptide pool (via ELISpot assay).

The results shown in Table 8 indicate comparable responses with respect to the generation of anti-gag antibodies. The frequencies of gag-specific T cells in

peripheral blood assummarized in Table 9 demonstrate a strong cellular immune response generated after a single dose with the new construct MRK Ad5 HIV-1 gag. The responses are also boostable with second dose of the same vector. The vector is also able to induce CD8+ T cell responses (as evident by remaining spot counts after CD4+ depletion of PBMCs) which are responsible for cytotoxic activity.

Table 8 Anti-p24 antibody titers (in mMU/mL) in rhesus macaques immunized with

gag-expressing adenovectors (Protocol HIV203).

gag-expressing adenovecto								
Vaccine	Pre	Wk 4	Wk 8_	Wk 12	Wk 16	Wk 20	Wk 25	Wk 28
MR KAd5gag <sup>a</sup> , 10^11 vp								
97N010	<10	118	5528_	11523	7062	21997	ND	51593
97N116	<10	62	772_	1447	1562	2174	ND	20029
98X007	<10	66	3353	6156	6845	3719	ND	24031
MRKAd5gag, 10^9 vp								
97N120	<10_	51	204	318	366	482	ND	6550
97N144	<10	18	118	274	706	888	ND	7136
98X008	<10	15	444	386	996	1072	ND	12851
Ad5gag <sup>b</sup> , Clinical Lot, 10^11 vp								
97X001	<10	87	2579	4718	7174	7250	ND	69226
97N146	<10	72	3604	7380	7526	18906	ND	60283
98X009	<10	78	4183	3946	3124	6956	ND	26226
Ad5gag, Clinical Lot, 10^9 vp								
97N020	<10	<10	143	371	390	1821	ND	17177
97X003	<10	<10	39	93	156	596	ND_	2053
98X012	<10	81	342	717	956	1558	ND	11861
MRKActigag (hCMV, bGHpA, E3+)		L	<u> </u>	<b>.</b>				
barlginal Actigag vector (hCMV/Intra	n A bGHp	4, E3-), lott	FN0001_					
ND, not determined		<u></u>		<u> </u>			<u> </u>	L

Table 9. Number of gag-specific T cells per million peripheral blood mononuclear cells (PBMCs) in rhesus monkeys immunized with gag-expressing adenovectors. Also included are those frequencies in PBMCs depleted of CD4<sup>+</sup> T cells.

Grp#	Vaccination	Monkey ID	1=4	Wk	T=6	Wk	[=]	l Wk	T=10	6 Wk	ĭ=2	5 Wk	T=2	Wk_
<b></b>	T=0,4,25 wks		Mediq	Gog H <sup>b</sup>	Media	Gog H	Media	Gog H	Media	Gog H	Media	Gog H	Media	Gog H
			١.			l	_	,,,,,	ا ہ ا	1174	3	775	4	1074
1	MRKAc5gcg	97N010	6	89	0	395	0	1058	U	11/4	ő	76	a	594
	10411 VD	97N010(CD4-)	4	38	' . ا	609	3	993 534	4	395	1 ,	261	ŏ	408
		97N116	1	396	1	609	0		1 4	373	١.	184	ŏ	666
		97N116(CD4-)	111	676			0	593	Ι.,	۱		1588	ő	2113
		98X007	10	579	0	1304	3	2193	1	2118	3			1278
		98X007(CD4-)	20	965			0	2675		ŀ	0	1656	0	12/6
2	MRKAd5ccc	97N120	5	275	1	249	4	141	4	119	9	206	4	219
_	10/9 VD	97N120(CD4-)	111	170		ŀ	0	85	l I	!	0	75	1	219
	·	97N144	3	236	6	438	1	318	3	256	וו	98	5	373
		97N144(CD4-)	6	148	l i	ł	0	285	ŀ		ND	NO	0	625
		98X008	4	368	1	1090	3	891	4	673	3	473	5	735
		98X008(CD4-)	14	696		1	0	1175	ł		٥	391	4	848
3	Actigog clinical lat	97X001	6	261	1	485	0	817	0	12205	1	894	0	1858
3	10^11 vo	97X001(CD4-)	10	283	٠		3	996			0	1010	0	1123
	,,	97N146	3	150	1	465 i	0	339	1	1272	3	1238	3	1785
		97N146(CD4-)	ه ا	133		[	0	370			0	654	0	971
		98X009	lo	93	3	339	3	559	0	896	] ]	384	0	1748
		98X009(CD4-)	0	73			0	333			0	225	0	644
4	Actigag dinical lat	97N020	3	30	1	101	0	66	0	36	0	26	0	41
7	10/9 VD	97N020(CD4-)	10	29		J	0	15			0	1	0	16
	.5 7 12	97X003	4	68	5	134	0	18	1	38	4	38	6	81
		97X003(CD4-)	۱,	40			0	6	1	i	0	4	0	19
		98X012	5	95	3	54	1	34	0	18	0	20	1 1	121
		98X012(CD4-)	ii	70	i	1	O	11			0	8	0	41
5	Nave	96R041	6	8	1	1	0	0	0	0	0	0	1	0
-		053F	14	18	5	16.	20	14	19	15	10	15	24	9

Based on either 4x10/6 or 2x10/6 cells per well (depending on spot density)

ND, not determined

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"mock or no peptide control

Pool of 20-capeptides overlanding by 10 caland encompassing the gag sequence

The adenovectors described herein and, particularly, MRK Ad5 HIV-1 gag, represent very promising HIV-gag adenovectors with respect to their enhanced growth characteristics in both serum and, more importantly, in serum-free media conditions. In comparison with the current HIV-1 gag adenovector construct, MRK Ad5 HIV-1 gag shows a 5-10 fold increased amplification rate. We have shown that it is genetically stable at passage 21. This construct is able to generate significant cellular immune responses *in vivo* even at a relatively low dose of 10^9 vp. The potency of the MRKAd5gag construct is comparable to, if not better than the original HIV-1gag vector as shown in this rhesus monkey study.

# EXAMPLE 17 CODON OPTIMIZED HIV-1 POL AND CODON OPTIMZED HIV-1 POL MODIFICATIONS

The open reading frames for the various synthetic *pol* genes disclosed herein comprise coding sequences for the reverse transcriptase (or RT which consists of a polymerase and RNase H activity) and integrase (IN). The protein sequence is based

on that of Hxb2r, a clonal isolate of IIIB; this sequence has been shown to be closest to the consensus clade B sequence with only 16 nonidentical residues out of 848 (Korber, et al., 1998, Human retroviruses and AIDS, Los Alamos National Laboratory, Los Alamos, New Mexico). The skilled artisan will understand after review of this specification that any available HIV-1 or HIV-2 strain provides a potential template for the generation of HIV pol DNA vaccine constructs disclosed herein. It is further noted that the protease gene is excluded from the DNA vaccine constructs of the present invention to insure safety from any residual protease activity in spite of mutational inactivation. The design of the gene sequences for both wildtype (wt-pol) and inactivated pol (IA-pol) incorporates the use of human preferred ("humanized") codons for each amino acid residue in the sequence in order to maximize in vivo mammalian expression (Lathe, 1985, J. Mol. Biol. 183:1-12). As can be discerned by inspecting the codon usage in SEQ ID NOs: 1, 3, 5 and 7, the following codon usage for mammalian optimization is preferred: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which, as noted elsewhere in this specification, is hereby incorporated by reference. It is intended that the skilled artisan may use alternative versions of codon optimization or may omit this step when generating HIV pol vaccine constructs within the scope of the present invention. Therefore, the present invention also relates to non-codon optimized versions of DNA molecules and associated recombinant adenoviral HIV vaccines which encode the various wild type and modified forms of the HIV Pol protein disclosed herein. However, codon optimization of these constructs is a preferred embodiment of this invention.

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A particular embodiment of this portion of the invention comprisies codon optimized nucleotide sequences which encode wt-pol DNA constructs (herein, "wtpol" or "wt-pol (codon optimized))" wherein DNA sequences encoding the protease (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1, the open reading frame being contained from an initiating Met residue at nucleotides 10-12 to a termination codon from nucleotides 2560-2562. SEQ ID NO:1 is as follows: AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC

ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG

	GAAATCTGCA	CTGAGATGGA	GAAGGAGGC	AAAATCTCCA	AGATTGGCCC	CGAGAACCCC
	TACAACACCC	CTGTGTTTGC	CATCAAGAAG	AAGGACTCCA	CCAAGTGGAG	GAAGCTGGTG
	GACTTCAGGG	AGCTGAACAA	GAGGACCCAG	GACTTCTGGG	AGGTGCAGCT	GGGCATCCCC
	CACCCCGCTG	GCCTGAAGAA	GAAGAAGTCT	GTGACTGTGC	TGGATGTGGG	GGATGCCTAC
5	TTCTCTGTGC	CCCTGGATGA	GGACTTCAGG	AAGTACACTG	CCTTCACCAT	CCCCTCCATC
	AACAATGAGA	CCCCTGGCAT	CAGGTACCAG	TACAATGTGC	TGCCCCAGGG	CTGGAAGGGC
	TCCCCTGCCA	TCTTCCAGTC	CTCCATGACC	AAGATCCTGG	AGCCCTTCAG	GAAGCAGAAC
	CCTGACATTG	TGATCTACCA	GTACATGGAT	GACCTGTATG	TGGGCTCTGA	CCTGGAGATT
	GGGCAGCACA	GGACCAAGAT	TGAGGAGCTG	AGGCAGCACC	TGCTGAGGTG	GGGCCTGACC
10	ACCCCTGACA	AGAAGCACCA	GAAGGAGCCC	CCCTTCCTGT	GGATGGGCTA	TGAGCTGCAC
	CCCGACAAGT	GGACTGTGCA	GCCCATTGTG	CTGCCTGAGA	AGGACTCCTG	GACTGTGAAT
	GACATCCAGA	AGCTGGTGGG	CAAGCTGAAC	TGGGCCTCCC	AAATCTACCC	TGGCATCAAG
	GTGAGGCAGC	TGTGCAAGCT	GCTGAGGGGC	ACCAAGGCCC	TGACTGAGGT	GATCCCCCTG
	ACTGAGGAGG	CTGAGCTGGA	GCTGGCTGAG	AACAGGGAGA	TCCTGAAGGA	GCCTGTGCAT
15	GGGGTGTACT	ATGACCCCTC	CAAGGACCTG	ATTGCTGAGA	TCCAGAAGCA	GGGCCAGGGC
÷	CAGTGGACCT	ACCAAATCTA	CCAGGAGCCC	TTCAAGAACC	TGAAGACTGG	CAAGTATGCC
	AGGATGAGGG	GGGCCCACAC	CAATGATGTG	AAGCAGCTGA	CTGAGGCTGT	GCAGAAGATC
	ACCACTGAGT	CCATTGTGAT	CTGGGGCAAG	ACCCCCAAGT	TCAAGCTGCC	CATCCAGAAG
	GAGACCTGGG	AGACCTGGTG	GACTGAGTAC	TGGCAGGCCA	CCTGGATCCC	TGAGTGGGAG
20	TTTGTGAACA	CCCCCCCCT	GGTGAAGCTG	TGGTACCAGC	TGGAGAAGGA	GCCCATTGTG
	GGGGCTGAGA	CCTTCTATGT	GGATGGGGCT	GCCAACAGGG	AGACCAAGCT	GGGCAAGGCT
	GGCTATGTGA	CCAACAGGGG	CAGGCAGAAG	GTGGTGACCC	TGACTGACAC	CACCAACCAG
	AAGACTGAGC	TCCAGGCCAT	CTACCTGGCC	CTCCAGGACT	CTGGCCTGGA	GGTGAACATT
	GTGACTGACT	CCCAGTATGC	CCTGGGCATC	ATCCAGGCCC	AGCCTGATCA	GTCTGAGTCT
25	GAGCTGGTGA	ACCAGATCAT	TGAGCAGCTG	ATCAAGAAGG	AGAAGGTGTA	CCTGGCCTGG
	GTGCCTGCCC	ACAAGGGCAT	TGGGGGCAAT	GAGCAGGTGG	ACAAGCTGGT	GTCTGCTGGC
	ATCAGGAAGG	TGCTGTTCCT	GGATGGCATT	GACAAGGCCC	AGGATGAGCA	TGAGAAGTAC
	CACTCCAACT	GGAGGGCTAT	GGCCTCTGAC	TTCAACCTGC	CCCCTGTGGT	GGCTAAGGAG
	ATTGTGGCCT	CCTGTGACAA	GTGCCAGCTG	AAGGGGGAGG	CCATGCATGG	GCAGGTGGAC
30	TGCTCCCCTG	GCATCTGGCA	GCTGGACTGC	ACCCACCTGG	AGGGCAAGGT	GATCCTGGTG
	GCTGTGCATG	TGGCCTCCGG	CTACATTGAG	GCTGAGGTGA	TCCCTGCTGA	GACAGGCCAG
1	GAGACTGCCT	ACTTCCTGCT	GAAGCTGGCT	GGCAGGTGGC	CTGTGAAGAC	CATCCACACT
	GACAATGGCT	CCAACTTCAC	TGGGGCCACA	GTGAGGGCTG	CCTGCTGGTG	GGCTGGCATC
	AAGCAGGAGT	TTGGCATCCC	CTACAACCCC	CAGTCCCAGG	GGGTGGTGGA	GTCCATGAAC
35	AAGGAGCTGA	AGAAGATCAT	TGGGCAGGTG	AGGGACCAGG	CTGAGCACCT	GAAGACAGCT
	GTGCAGATGG	CTGTGTTCAT	CCACAACTTC	AAGAGGAAGG	GGGGCATCGG	GGGCTACTCC

GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT
GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ
ID NO:1).

The open reading frame of the wild type pol construct disclosed as SEQ ID NO:1 contains 850 amino acids, disclosed herein as SEQ ID NO:2, as follows: Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys 10 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp 15 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln 20 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile 25 Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys 30 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu 35 Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala

Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys 10 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val 15 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly 20 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp 25 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:2).

The present invention especially relates to an adenoviral vector vaccine which comprises a codon optimized HIV-1 DNA pol construct wherein, in addition to deletion of the portion of the wild type sequence encoding the protease activity, a combination of active site residue mutations are introduced which are deleterious to HIV-1 pol (RT-RH-IN) activity of the expressed protein. Therefore, the present invention preferably relates to an adenoviral HIV-1 DNA pol-based vaccine wherein the construct is devoid of DNA sequences encoding any PR activity, as well as containing a mutation(s) which at least partially, and preferably substantially, abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant which is part and parcel of an adenoviral vector vaccine may include but is not limited to a mutated

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DNA molecule comprising at least one nucleotide substitution which results in a point mutation which effectively alters an active site within the RT, RNase and/or IN regions of the expressed protein, resulting in at least substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at 10 least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEQ ID NO:4, Figure 15 17A-C) which has no PR, RT, RNase or IN activity, wherein three such point mutations reside within each of the RT, RNase and IN catalytic domains. Therefore, an especially preferred exemplification is an adenoviral vaccine which comprises, in an appropriate fashion, a DNA molecule which encodes IA-pol, which contains all nine mutations as shown below in Table 1. An additional preferred amino acid residue for substitution is Asp551, localized within the RNase domain of Pol. Any 20 combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the preferred mutation is a point mutation resulting in a substitution of the wild type amino acid with an alternative amino acid residue.

#### Table 1

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	wt aa	aa residue	mutant aa	enzyme function
	Asp	112	Ala	RT
	Asp	187	Ala	RT
30	Asp	188	Ala	RT
	Asp	445	Ala	RNase H
	Glu	480	Ala	RNase H
	Asp	500	Ala	RNase H
	Asp	626	Ala	IN
35	Asp	678	Ala	IN
	Glu	714	Ala	IN

It is preferred that point mutations be incorporated into the IApol mutant adenoviral vaccines of the present invention so as to lessen the possibility of altering epitopes in and around the active site(s) of HIV-1 Pol.

To this end, SEQ ID NO:3 discloses the nucleotide sequence which codes for a codon optimized pol in addition to the nine mutations shown in Table 1, disclosed as follows, and referred to herein as "IApol":

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AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG 10 GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGCTGTGGG GGATGCCTAC TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC 15 TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC CCTGACATTG TGATCTACCA GTACATGGCT GCCCTGTATG TGGGCTCTGA CCTGGAGATT GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC CCCGACAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG 20 GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC AGGATGAGGG GGGCCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC 25 ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG TTTGTGAACA CCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG GGGGCTGAGA CCTTCTATGT GGCTGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG 30 AAGACTGCCC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT GTGACTGCCT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC ATCAGGAAGG TGCTGTTCCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC 35 CACTCCAACT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG

ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC
TGCTCCCCTG GCATCTGGCA GCTGGCCTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG
GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG
GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT
GCCAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC
AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGC CTCCATGAAC
AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT
GTGCAGATGG CTGTGTTCAT CCACAACTTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC
GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTT TGGTGATCCA GGACAACTCT
GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID
NO:3).

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15 In order to produce the IA-pol-based adenoviral vaccines of the present invention, inactivation of the enzymatic functions was achieved by replacing a total of nine active site residues from the enzyme subunits with alanine side-chains. As shown in Table 1, all residues that comprise the catalytic triad of the polymerase, namely Asp112, Asp187, and Asp188, were substituted with alanine (Ala) residues 20 (Larder, et al., Nature 1987, 327: 716-717; Larder, et al., 1989, Proc. Natl. Acad. Sci. 1989, 86: 4803-4807). Three additional mutations were introduced at Asp445, Glu480 and Asp500 to abolish RNase H activity (Asp551 was left unchanged in this IA Pol construct), with each residue being substituted for an Ala residue, respectively (Davies, et al., 1991, Science 252:, 88-95; Schatz, et al., 1989, FEBS Lett. 257: 311-25 314; Mizrahi, et al., 1990, Nucl. Acids. Res. 18: pp. 5359-5353). HIV pol integrase function was abolished through three mutations at Asp626, Asp678 and Glu714. Again, each of these residues has been substituted with an Ala residue (Wiskerchen, et al., 1995, J. Virol. 69: 376-386; Leavitt, et al., 1993, J. Biol. Chem. 268: 2113-2119). Amino acid residue Pro3 of SEQ ID NO:4 marks the start of the RT gene. 30 The complete amino acid sequence of IA-Pol is disclosed herein as SEO ID NO:4 and Figure 17A-C, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg

Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln 10 Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr 15 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp 20 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly 25 Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys 30 Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln 35 Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly

Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:4).

As noted above, it will be understood that any combination of the mutations disclosed above may be suitable and therefore be utilized as an IA-pol-based adenoviral HIV vaccine of the present invention, either when administered alone or in a combined modality regime and/or a prime-boost regimen. For example, it may be possible to mutate only 2 of the 3 residues within the respective reverse transcriptase, RNase-H, and integrase coding regions while still abolishing these enzymatic activities. However, the IA-pol construct described above and disclosed as SEQ ID NO:3, as well as the expressed protein (SEQ ID NO:4;) is preferred. It is also preferred that at least one mutation be present in each of the three catalytic domains.

Another aspect of this portion of the invention are codon optimized HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA (tissue-type plasminogen activator) or by a leader peptide such as is found in highly expressed mammalian proteins such as immunoglobulin leader peptides. Any functional leader peptide may be tested for efficacy. However, a preferred embodiment of the present invention, as with HIV-1 Nef constructs shown herein, is to provide for a HIV-1 Pol mutant adenoviral vaccine construction wherein the pol coding region or a portion thereof is operatively linked to a leader peptide, preferably a leader peptide from human tPA. In other words, a codon optimized HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) may also comprise a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. As noted in Figure 16A-B, a DNA vector which may be utilized to practice the present invention may be modified by known recombinant DNA methodology to contain a leader signal

peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Pol protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Pol protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Pol protein of interest, including but not limited to a HIV-1 Pol protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17). Therefore, another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

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To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region (herein, "tPA-wt-pol"). A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame being contained from an initiating Met residue at nucleotides 8-10 to a termination codon from nucleotides 2633-2635. SEQ ID NO:5 is as follows:

25 GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA
GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGATGT
GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC
CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT

35 CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GATGACCTGT ATGTGGGCTC
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG

GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT 10 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGATGGG GCTGCCAACA GGGAGACCAA GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA CACCACCAAC CAGAAGACTG AGCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT 15 GGAGGTGAAC ATTGTGACTG ACTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA 20 TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGAC TGCACCCACC TGGAGGGCAA GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA GACCATCCAC ACTGACAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG 25 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT GGAGTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG 30 GAACCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC GGGCAGATCT (SEQ ID NO:5).

The open reading frame of the wild type tPA-pol construct disclosed as SEQ ID NO:5 contains 875 amino acids, disclosed herein as SEQ ID NO:6, as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly

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Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro 10 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp 15 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile 20 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe 25 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu 30 Thr Asp Thr Thr Asn Gln Lys Thr.Glu.Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu 35 Ala, Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile

Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val 10 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly 15 Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:6).

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The present invention also relates to a codon optimized HIV-1 Pol mutant contained within a recombinant adenoviral vector such as IA-Pol (SEQ ID NO:4) which comprises a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. Any such adenoviral-based HIV-1 DNA pol mutant disclosed in the above paragraphs is suitable for fusion downstream of a leader peptide, such as a leader peptide including but not limited to the human tPA leader sequence. Therefore, any such leader peptide-based HIV-1 pol mutant construct may include but is not limited to a mutated DNA molecule which effectively alters the catalytic activity of the RT, RNase and/or IN region of the expressed protein, resulting in at least substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at least one point mutation which alters the active site and catalytic activity within the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely

comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion junction as shown in Figure 18. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8. To this end, SEQ ID NO:7 discloses the nucleotide sequence which codes for a human tPA leader fused to the IA Pol protein comprising the nine mutations shown in Table 1 (herein, "tPA-opt-IApol"). The open 10 reading frame begins with the initiating Met (nucleotides 8-10) and terminates with a "TAA" codon at nucleotides 2633-2635. The nucleotide sequence encoding tPA-IAPol is also disclosed as follows: GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA 15 GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA GCTGGGCATC CCCCACCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGCTGT 20 GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GCTGCCCTGT ATGTGGGCTC TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG .25 GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA 30 -GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT 35 CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA

GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGCTGGG GCTGCCAACA GGGAGACCAA

GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA CACCACCAAC CAGAAGACTG CCCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT GGAGGTGAAC ATTGTGACTG CCTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGCC TGCACCCACC TGGAGGGCAA 10 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA GACCATCCAC ACTGCCAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT GGCCTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT 15 CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG GAACCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA 20 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC GGGCAGATCT (SEQ ID NO:7).

The open reading frame of the tPA-IA-pol construct disclosed as SEQ ID NO:7 contains 875 amino acids, disclosed herein as tPA-IA-Pol and SEQ ID NO:8, as follows:

25 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu 30 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr

Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu 10 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr 15 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala 20 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln beu Ile Lys Lys Glu Lys Val Tyr beu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile 25 Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu 30 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val 35 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe

Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:8).

#### **EXAMPLE 18**

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### CODON OPTIMIZED HIV-1 NEF AND CODON OPTIMIZED **HIV-1 NEF MODIFICATIONS**

Codon optimized version of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed - 15 December 15, 2000, both documents which are hereby incorporated by reference. As disclosed within the above-mentioned documents, particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH2-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEO ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which

encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16.

As disclosed in the above-identified documents (U.S. Application Serial No. 09/738,782 and PCT International Application PCT/US00/34162) and reiterated herein, the following nef-based nucleotide and amino acid sequences which comprise the respective open reading frame are as follows:

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1. The nucleotide sequence of the codon optimized version of HIV-1 jrfl nef gene is disclosed herein as SEQ ID NO:9, as shown herein:

GATCTGCCAC CATGGGCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA

GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG

CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA

ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG

GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC

TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC

AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT

ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC

CCGTGGAGCC CGAGAAGGTG GAGGAGCCCA ACGAGGGCGA GAACAACTGC CTGCTGCACC

CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT

CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT

AAAGCCCGGG C (SEQ ID NO:9).

Preferred codon usage is as follows: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which is hereby incorporated by reference. See also Figure 19A-B for a comparion of wild type vs. codon optimized nucleotides comprising the open reading frame of HIV-Nef.

The open reading frame for SEQ ID NO:9 above comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The open reading frame of SEQ ID NO:9 provides for a 216 amino acid HIV-1 Nef protein expressed through utilization of a codon optimized DNA vaccine vector. The 216 amino acid HIV-1 Nef (jfrl) protein is disclosed herein as SEQ ID NO:10, and as follows:

Met Gly Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val

Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Gly Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:10).

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HIV-1 Nef is a 216 amino acid cytosolic protein which associates with the inner surface of the host cell plasma membrane through myristylation of Gly-2 (Franchini et al., 1986, Virology 155: 593-599). While not all possible Nef functions have been elucidated, it has become clear that correct trafficking of Nef to the inner plasma membrane promotes viral replication by altering the host intracellular environment to facilitate the early phase of the HIV-1 life cycle and by increasing the infectivity of progeny viral particles. In one aspect of the invention regarding codon-optimized, protein-modified polypeptides, the nef-encoding region of the adenovirus vector of the present invention is modified to contain a nucleotide sequence which encodes a heterologous leader peptide such that the amino terminal region of the expressed protein will contain the leader peptide. The diversity of function that typifies eukaryotic cells depends upon the structural differentiation of their membrane boundaries. To generate and maintain these structures, proteins must be transported from their site of synthesis in the endoplasmic reticulum to predetermined destinations throughout the cell. This requires that the trafficking proteins display sorting signals that are recognized by the molecular machinery responsible for route selection located at the access points to the main trafficking pathways. Sorting decisions for most proteins need to be made only once as they traverse their biosynthetic pathways since their final destination, the cellular location at which they perform their function, becomes their permanent residence. Maintenance of intracellular integrity depends in part on the selective sorting and accurate transport of proteins to their correct destinations. Defined sequence motifs exist in proteins which can act as 'address labels'. A number of sorting signals have

been found associated with the cytoplasmic domains of membrane proteins. An effective induction of CTL responses often required sustained, high level endogenous expression of an antigen. As membrane-association via myristylation is an essential requirement for most of Nef's function, mutants lacking myristylation, by glycine-to-alanine change, change of the dileucine motif and/or by substitution with a tpa leader sequence as described herein, will be functionally defective, and therefore will have improved safety profile compared to wild-type Nef for use as an HIV-1 vaccine component.

In another embodiment of this portion of the invention, either the DNA vector or the HIV-1 nef nucleotide sequence is modified to include the human tissue-specific plasminogen activator (tPA) leader. As shown in Figure 16A-B, a DNA vector may be modified by known recombinant DNA methodology to contain a leader signal peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Nef protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Nef protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Nef protein of interest, including but not limited to a HIV-1 Nef protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17).

It has been shown that myristylation of Gly-2 in conjunction with a dileucine motif in the carboxy region of the protein is essential for Nef-induced down regulation of CD4 (Aiken et al., 1994, Cell 76: 853-864) via endocytosis. It has also been shown that Nef expression promotes down regulation of MHCI (Schwartz et al., 1996, Nature Medicine 2(3): 338-342) via endocytosis. The present invention relates in part to DNA vaccines which encode modified Nef proteins altered in trafficking and/or functional properties. The modifications introduced into the adenoviral vector HIV vaccines of the present invention include but are not limited to additions, deletions or substitutions to the nef open reading frame which results in the expression of a modified Nef protein which includes an amino terminal leader peptide, modification or deletion of the amino terminal myristylation site, and modification or deletion of the dileucine motif within the Nef protein and which alter function within the infected host cell. Therefore, a central theme of the DNA molecules and recombinant adenoviral HIV vaccines of the present invention is (1)

host administration and intracellular delivery of a codon optimized nef-based adenoviral HIV vaccine; (2) expression of a modified Nef protein which is immunogenic in terms of eliciting both CTL and Th responses; and, (3) inhibiting or at least altering known early viral functions of Nef which have been shown to promote HIV-1 replication and load within an infected host. Therefore, the nef coding region may be altered, resulting in a DNA vaccine which expresses a modified Nef protein wherein the amino terminal Gly-2 myristylation residue is either deleted or modified to express alternate amino acid residues. Also, the nef coding region may be altered so as to result in a DNA vaccine which expresses a modified Nef protein wherein the dileucine motif is either deleted or modified to express alternate amino acid residues. In addition, the adenoviral vector HIV vaccines of the present invention also relate to an isolated DNA molecule, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175 and/or inclusion of a leader sequence.

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Therefore, specific Nef-based constructs further include the following, as exemplification's and not limitations. For example, the present invention relates to an adenoviral vector vaccine which encodes modified forms of HIV-1, an open reading frame which encodes a Nef protein which comprises a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl) is referred to herein as opt tpanef. The nucleotide sequence comprising the open reading frame of opt tpanef is disclosed herein as SEQ ID NO:11, as shown below:

CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG 25 GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCCTGCTGC ACCCCATGTC CCAGCACGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACTCCAAGCT GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCC (SEQ ID N0:11).

The open reading frame for SEQ ID NO:11 comprises an initiating methionine

residue at nucleotides 2-4 and a "TAA" stop codon from nucleotides 713-715. The open reading frame of SEQ ID NO:3 provides for a 237 amino acid HIV-1 Nef protein which comprises a tPA leader sequence fused to amino acids 6-216 of HIV-1 Nef, including the dileucine motif at amino acid residues 174 and 175. This 237 amino acid tPA/Nef (jfrl) fusion protein is disclosed herein as SEQ ID NO:12, and is shown as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val 10 Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp 15 Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His 20 Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:12). Therefore, this exemplified Nef protein, Opt tPA-Nef, contains both a tPA leader sequence as well as deleting the myristylation site of Gly-2A DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. 25

In another specific embodiment of the present invention, a DNA molecule is disclosed which encodes optimized HIV-1 Nef wherein the open reading frame of a recombinant adenoviral HIV vaccine encodes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175. This open reading frame is herein described as opt nef (G2A,LLAA) and is disclosed as SEQ ID NO:13, which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The nucleotide sequence of this codon optimized version of HIV-1 jrfl nef gene with the above mentioned modifications is disclosed herein as SEQ ID NO:13, as follows:

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GATCTGCCAC CATGGCCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCA ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCG CTGGTGCTTC AAGCTGGTGC CCGTGGAGCC CGAGAAGGGC ACGAGGGCCA ACGAGGGCGA GAACAACTGC GCCGCCCACC CCATGTCCCA GCACGGCATC GAGGACCCC AGGAGGGCGA GAACAACTGC GCCGCCCACC CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGAGGT GCTGGAGTGG AGGTTCGACT CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT AAAGCCCGGG C (SEQ ID NO:13).

The open reading frame of SEQ ID NO:13 encodes Nef (G2A,LLAA), disclosed herein as SEQ ID NO:14, as follows:

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15 Met Ala Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val 20 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro 25 Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys Ser (SEQ ID NO:14).

An additional embodiment of the present invention relates to another DNA molecule encoding optimized HTV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA) comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HTV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175 (Ala-195 and Ala-196 in this tPA-based fusion protein). The nucleotide

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sequence comprising the open reading frame of opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, as shown below:

CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG 5 GATGAGGAGG GCCGACCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA CTTCCTGAAG GAGAAGGGCG GCCTGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC CGGCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCGCCGCCC ACCCCATGTC CCAGCACGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACTCCAAGCT GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCCC (SEQ ID NO:15).

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The open reading frame of SEQ ID NO:7 encoding tPA-Nef (LLAA), disclosed herein as SEQ ID NO:16, is as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro 20 Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr 25 Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn 30 Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:16). An adenoviral vector of the present invention may comprise a DNA sequence, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a 35 deletion or substitution of Gly 2, a deletion of substitution of Leu 174 and Leu 175

and/or inclusion of a leader sequence. Therefore, partial or fully codon optimized DNA vaccine expression vector constructs are preferred since such constructs should result in increased host expression. However, it is within the scope of the present invention to utilize "non-codon optimized" versions of the constructs disclosed herein, especially modified versions of HIV Nef which are shown to promote a substantial cellular immune response subsequent to host administration.

Figure 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively.

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#### **EXAMPLE 19**

#### MRKAd5Pol Construction and Virus Rescue

Steps performed in the construction of the vectors, including the pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus plasmid denoted MRKAd5pol, is depicted in Figure 22. Briefly, the adenoviral shuttle vector for the full-length inactivated HIV-1 pol gene is as follows. The vector MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is a derivative of the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. The vector contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique Bg/II site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 (or MRKpAdHVE3) preplasmid. The vector, similar to the original shuttle vector contains the Pac1 site, extension to the packaging signal region, and extension to the pIX gene. The synthetic full-length codon-optimized HIV-1 pol gene was isolated directly from the plasmid pV1Jns-HIV-pol-inact(opt). Digestion of this plasmid with Bg/I II releases the pol

gene intact (comprising a codon optimized IA pol sequence as disclosed in SEQ ID NO:3). The pol fragment was gel purified and ligated into the MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) shuttle vector at the BgIII site. The clones were checked for the correct orientation of the gene by using restriction enzymes DraIII/Not1. A positive clone was isolated and named MRKpdel+hCMVmin+FL-pol+bGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdel+hCMVmin+FLpol+bGHpA(S) was digested with restriction enzymes Pac1 and Bst1107 I (or its isoschizomer, BstZ107 I) and then co-transformed into E. coli strain BJ5183 with linearized (Cla1 digested) adenoviral backbone plasmid, MRKpAd(E1-/E3+)Cla1. The resulting pre-plasmid originally named MRKpAd+hCMVmin+FLpol+bGHpA(S)E3+ is now referred to as "pMRKAd5pol". The genetic structure of the resulting pMRKAd5pol was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent E. coli XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the pol transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1pol adenoviral vector is shown in Figure 26 A-AO.

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Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5pol, was rescued as infectious virions in PER.C6® adherent monolayer cell culture. To rescue infectious virus, 12  $\mu$ g of pMRKAd5pol was digested with restriction enzyme PacI (New England Biolabs) and 3.3  $\mu$ g was transfected per 6 cm dish of PER.C6® cells using the calcium phosphate coprecipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech Inc.). PacI digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6® cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at  $\leq$  -60°C. This pol containing recombinant adenovirus is referred to herein as "MRKAd5pol". This recombinant adenovirus expresses an inactivated HIV-1 Pol protein as shown in SEQ ID NO:6.

#### **EXAMPLE 20**

#### MRKAd5Nef Construction and Virus Rescue

Construction of vector: shuttle plasmid and pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus

plasmid denoted MRKAd5nef, is depicted in Figure 23. Briefly, as shown in Example 19 above, the vector

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MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. It has been modified to contain the *Pac*1 site, extension to the packaging signal region, and extension to the pIX gene. It contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*11 site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 pre-plasmid. The synthetic full-length codon-optimized HIV-1 nef gene was isolated directly from the plasmid pV1Jns/nef (G2A,LLAA). Digestion of this plasmid with *Bgl*11 releases the pol gene intact, which comprises the nucleotide sequence as disclosed in SEQ ID NO:13. The nef fragment was gel purified and ligated into the

MRKpdelE1+CMVmin+BGHpA(str.) shuttle vector at the *Bgl*11 site. The clones were checked for correction orientation of the gene by using restriction enzyme *Sca*1. A positive clone was isolated and named MRKpdelE1hCMVminFL-nefBGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdelE1hCMVminFL-nefBGHpA(s) was digested with restriction enzymes *Pac*1 and *Bst*1107 I (or its isoschizomer, *Bst*Z107 I) and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla*1 digested) adenoviral backbone plasmid, MRKpAd(E1/E3+)Cla1. The resulting pre-plasmid originally named MRKpdelE1hCMVminFL-nefBGHpA(s) is now referred to as "pMRKAd5nef". The genetic structure of the resulting pMRKAd5nef was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the

of this pMRKAd5HIV-1nef adenoviral vector is shown in Figure 27A-AM.

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5nef, was rescued as infectious virions in PER.C6® adherent monolayer cell culture. To rescue infectious virus, 12 µg of pMRKAdnef was digested with restriction enzyme Pac1 (New England Biolabs) and 3.3 µg was transfected per 6 cm dish of PER.C6® cells using the calcium phosphate coprecipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech

nef transgene in transient transfection cell culture. The complete nucleotide sequence

Inc.). Pac1 digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6® cells. Infected cells and media were harvested 6-10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at  $\leq$  -60°C. This nef containing recombinant adenovirus is now referred to as "MRKAd5nef".

#### **EXAMPLE 21**

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Construction of Murine CMV Promoter Containing Shuttle Vectors for Inactivated Pol and Nef/G2A,LLAA

The murine CMV (mCMV) was amplified from the plasmid pMH4 (supplied by Frank Graham, McMaster University) using the primer set: mCMV (Not I) Forward: 5'-ATA AGA ATG CGG CCG CCA TAT ACT GAG TCA TTA GG-3' (SEQ ID NO: 20); mCMV (Bgl II)Reverse: 5'-AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C-3' (SEQ ID NO:21). The underlined nucleotides represent the Not I and the Bgl II sites respectively for each primer. This PCR amplicon was used for the construction of the mCMV shuttle vector containing the transgene in the E1 parallel orientation. The hCMV promoter was removed from the original shuttle vector (containing the hCMV-gag-bGHpA transgene in the E1 parallel orientation) by digestion with Not I and Bgl II. The mCMV promoter (Not I/Bgl II digested PCR product) was inserted into the shuttle vector in a directional manner. The shuttle vector was then digested with  $Bgl \coprod$  and the gag reporter gene ( $Bgl \coprod$  fragment) was re-inserted back into the shuttle vector. Several clones were screened for correct orientation of the reporter gene. For the construction of the mCMV-gag in the E1 antiparallel orientation, the mCMV promoter was amplified from the plasmid pMH4 using the following primer set: mCMV (Asc I) Forward: 5'- ATA AGA ATG GCG CGC CAT ATA CTG AGT CAT TAG G (SEQ ID NO:22); mCMV (Bgl II) Reverse: 5' AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C (SEQ ID NO:23). The underlined nucleotides represent the Asc I and Bgl II sites, respectively for each primer. The shuttle vector containing the hCMV-gag transgene in the E1 antiparallel orientation was digested with Asc1 and Bgl11 to remove the hCMV-gag portion of the transgene. The mCMV promoter (Asc1/Bgl11 digested PCR product) was inserted into the shuttle vector in a directional manner. The vector was then digested with Bgl11 and the gag reporter gene (Bgl11 fragment) was re-inserted. Several clones were screened for correct orientation of the reporter gene. For each of the full length IA pol and full length nef/G2A,LLAA genes, cloning was performed using the unique

 $Bgl \ \Pi$  site within the mCMV-bGHpA shuttle vector. The pol and nef genes were excised from their respective pV1Jns plasmids by  $Bgl \ \Pi$  digestion.

#### **EXAMPLE 22**

Construction of mCMV Full Length Inactivated Pol and Full Length nef/G2A.LLAA Adenovectors

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Each of these transgenes of Example 21 were inserted into the modified shuttle vector in both the E1 parallel and E1 anti-parallel orientations. *Pac1* and *BstZ110I* digestion of each shuttle vector was performed and each specific transgene fragment containing the flanking Ad5 sequences was isolated and co-transformed with *Cla I* digested MRKpAd5(E3+) or MRKpAd5(E3-) adenovector plasmids via bacterial homologous recombination in BJ5183 *E. coli* cells. Recombinant preplasmid adenovectors containing the various transgenes in both the E3- and E3+ versions (and in the E1 parallel and E1 antiparallel orientations) were subsequently prepared in large scale following transformation into XL-1 Blue *E. coli* cells and analyzed by restriction analysis and sequencing.

#### **EXAMPLE 23**

Construction of hCMV-tpa-nef (LLAA) Adenovector

The tpa-nef gene was amplified out from GMP grade pV1Ins-tpanef (LLAA) vector using the primer sets: Tpanef (BamHI) F 5'-ATT GGA TCC ATG GAT GCA ATG AAG AGA GGG (SEQ ID 24); Tpanef (BamHI) R 5'-ATA GGA TCC TTA GCA GTC CTT GTA GTA CTC G (SEQ ID NO:25). The resulting PCR product was digested with BamHI, gel purified and cloned into the Bgl II site of MRKAd5CMV-bGHpA shuttle vector (Bgl II digested and calf intestinal phosphatase treated). Clones containing the tpanef (LLAA) gene (see SEQ ID NO:15 for complet coding region) in the correct orientation with respect to the hCMV promoter were selected following Sca I digestion. The resulting MRKAd5tpanef shuttle vector was digested with Pac I and Bst Z1101 and cloned into the E3+ MRKAd5 adenovector via bacterial homologous recombination techniques.

#### **EXAMPLE 24**

Immunogenicity of MRKAd5pol and MRKAd5nef Vaccine

Materials and Methods - Rodent Immunization - Groups of N=10 BALB/c

mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-IApol

(E3+) at either 10^7 vp and 10^9 vp; and (2) MRKAd5hCMV-IApol (E3-) at either

10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively. For all rodent immunizations, the Ad5 vectors were diluted in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl2, 0.005% polysorbate 80, pH 8.0. The total dose was injected to both quadricep muscles in 50 μL aliquots using a 0.3-mL insulin syringe with 28-1/2G needles (Becton-Dickinson, Franklin Lakes, NJ).

Groups of N=10 C57/BL6 mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively.

Non-human Primate immunization - Cohorts of 3 rhesus macaques (2-3 kg) were vaccinated with the following Ad vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10^9 vp and 10^11 vp dose; and (2) MRKAd5hCMV-IApol (E3-) at either 10^9 vp and 10^11 vp; (3) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp; and (4) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp. The vaccine was administered to chemically restrained monkeys (10 mg/kg ketamine) by needle injection of two 0.5 mL aliquots of the Ad vectors (in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl<sub>2</sub>, 0.005% polysorbate 80, pH 8.0) into both deltoid muscles. The animals were immunized twice at a 4 week interval (T=0, 4 weeks).

Murine anti-RT and anti-nef ELISA - Anti-RT titers were obtained following standard secondary antibody-based ELISA. Maxisorp plates (NUNC, Rochester, NY) were coated by overnight incubation with 100 μL of 1 μg/mL HIV-1 RT protein (Advanced Biotechnologies, Columbia, MD) in PBS. For anti-nef ELISA, 100 uL of 1 ug/mL HIV-1 nef (Advanced Biotechnologies, Columbia, MD) was used to coat the plates. The plates were washed with PBS/0.05% Tween 20 using Titertek MAP instrument (Hunstville, AL) and incubated for 2 h with 200 μL/well of blocking solution (PBS/0.05% tween/1% BSA). An initial serum dilution of 100-fold was performed followed by 4-fold serial dilution. 100-μL aliquots of serially diluted samples were added per well and incubated for 2 h at room temperature. The plates

were washed and 100  $\mu$ L of 1/1000-diluted HRP-rabbit anti-mouse IgG (ZYMED, San Francisco, CA) were added with 1 h incubation. The plates were washed thoroughly and soaked with 100  $\mu$ L 1,2-phenylenediamine dihydrochloride/hydrogen peroxide (DAKO, Norway) solution for 15 min. The reaction was quenched by adding 100  $\mu$ L of 0.5M H<sub>2</sub>SO4 per well. OD<sub>492</sub> readings were recorded using Titertek Multiskan MCC/340 with S20 stacker. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value of greater than or equal to 0.1 OD<sub>492</sub> (2.5 times the background value).

Non-human primate and murine ELIspot assays - The enzyme-linked immuno-spot (ELISpot) assay was utilized to enumerate antigen-specific INFγ-secreting cells from mouse spleens (Miyahira, et al.1995, J. Immunol. Methods 181:45-54) or macaque PBMCs. Mouse spleens were pooled from 5 mice/cohort and single cell suspensions were prepared at  $5\times10^6$ /mL in complete RPMI media (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL streptomycin, 10 mM Hepes, 50 uM  $\beta$ -ME). Rhesus PBMCs were prepared from 8-15 mL of heparinized blood following standard Ficoll gradient separation (Coligan, et al, 1998, Current Protocols in Immunology. John Wiley & Sons, Inc.). Multiscreen opaque plates (Millipore, France) were coated with 100  $\mu$ L/well of either 5  $\mu$ g/mL purified rat anti-mouse IFN- $\gamma$  IgG1, clone R4-6A2 (Pharmingen, San Diego, CA), or 15 ug/mL mouse anti-human IFN- $\gamma$  IgG2a (Cat. No. 1598-00, R&D Systems, Minneapolis, MN) in PBS at 4°C overnight for murine or monkey assays, respectively. The plates were washed with PBS/penicillin/streptomycin and blocked with 200  $\mu$ L/well of complete RPMI media for 37 °C for at least 2 h.

To each well, 50 μL of cell samples (4-5x10<sup>5</sup> cells per well) and 50 μL of the antigen solution were added. To the control well, 50 μL of the media containing DMSO were added; for specific responses, either selected peptides or peptide pools (4 ug/mL per peptide final concentration) were added. For BALB/c mice immunized with the pol constructs, stimulation was conducted using a pool of CD4<sup>+</sup>-epitope containing 20-mer peptides (aa21-40, aa411-430, aa641-660, aa731-750, aa771-790) or a pool of CD8<sup>+</sup>-epitope containing peptides (aa201-220, aa311-330, aa781-800). For C57/BL6 mice immunized with the nef construct, either aa51-70 (CD8<sup>+</sup> T cell epitope) or aa81-100 (CD4<sup>+</sup>) peptide derived from the nef sequence was added for specific stimulation. In monkeys, the responses against pol were evaluated using two pools (L and R) of 20-aa peptides that encompass the entire pol sequence and overlap by 10 amino acids. In monkeys vaccinated with the nef constructs, a single pool containing 20-mer peptides covering the entire HIV-1 nef sequence and overlapping

by 10 aa was used. Each sample/antigen mixture was performed in triplicate wells for murine samples or in duplicate wells for rhesus PBMCs. Plates were incubated at 37°C, 5% CO<sub>2</sub>, 90% humidity for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and incubated with 100 μL/well of either 1.25 μg/mL biotin-conjugated rat anti-mouse IFN-γ mAb, clone XMG1.2 (Pharmingen) or of 0.1 ug/mL biotinylated anti-human IFN-gamma goat polyclonal antibody (R&D Systems) at 4°C overnight. The plates were washed and incubated with 100 μL/well 1/2500 dilution of strepavidin-alkaline phosphatase conjugate (Pharmingen) in PBS/0.005% Tween/5% FBS for 30 min at 37 °C. Spots were developed by incubating with 100 μL/well 1-step NBT/BCIP (Pierce Chemicals) for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each well was determined using a dissecting microscope and the data normalized to 10<sup>6</sup> cell input.

Non-human Primate anti-RT ELISA - The pol-specific antibodies in the monkeys were measured in a competitive RT EIA assay, wherein sample activity is determined by the ability to block RT antigen from binding to coating antibody on the plate well. Briefly, Maxisorp plates were coated with saturating amounts of pol positive human serum (#97111234). 250 uL of each sample is incubated with 15 uL of 266 ng/mL RT recombinant protein (in RCM 563, 1% BSA, 0.1% tween, 0.1% NaN<sub>3</sub>) and 20 uL of lysis buffer (Coulter p24 antigen assay kit) for 15 min at room temperature. Similar mixtures are prepared using serially diluted samples of a standard and a negative control which defines maximum RT binding. 200 uL/well of each sample and standard were added to the washed plate and the plate incubated 16-24 h at room temperature. Bound RT is quantified following the procedures described in Coulter p24 assay kit and reported in milliMerck units per mL arbitrarily defined by the chosen standard.

Results - Rodent Studies - BALB/c mice (n=5 mice/cohort) were immunized once or twice with varying doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). At 3 weeks after the second dose, Anti-pol IgG levels were determined by an ELISA assay using RT as a surrogate antigen. Cellular response were quantified via IFNγ ELISpot assay against pools of pol-epitope containing peptides. The results of these assays are summarized in Table 10. The results indicate that the mouse vaccinees exhibited detectable anti-RT IgGs with an adenovector dose as low as 10^7 vp. The humoral responses are highly dose-dependent and are boostable with a second immunization. One or two doses of either pol vectors elicit high frequencies of antigen-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells; the responses are weakly dose-dependent but are boostable with a second immunization.

Table 10. Immunogenicity of MRKAd5pol Vectors in BALB/c mice.

				Ал	ti-RT IgG Tite	are,	S	FC/10^6 cell	ls°
Gronb	Vaccine	Dose	No. of Doses	GMT	+SE	-SE	Medium	CD4+ peptide pool	CD8+ peptide pool
1	MRKAd5hCMVFLpol (E3+)	10^7 vp	2 1	310419 919	301785 372	153020 265	1(1) 1(1)	75(4) 72(9)	2313(67) 533(41)
2	MRKAd5hCMVFLpol (E3+)	10^9 vp	2 1	1638400° 713155	0 528520	0 303555	2(2) 1(1)	114(9) 48(7)	2063(182) 733(89)
3	MRKAÆhCMVFLpol (E3-)	10^7 vp	2 1	310419 6400	386218 14013	172097 4393	0(0) 10(8)	223(7) 141(21)	2607(27) 409(28)
4	MRKAd5hCMVFLpol (E3-)	10^9 vp	2 1	1638400 <sup>b</sup> 1241675 <sup>b</sup>	0 396725	0 300661	1(1) 0(0)	160(13) 39(13)	2385(11) 833(83)
5	Naïve	none	none	57	9	7	9(2)	11(4)	10(1)

<sup>a</sup>GMT, geometric mean liter of the cohort of 5 mice; SE, standard error of the gemetric mean

<sup>b</sup>Near or at the upper limit of the serial dilution; hence, could be greater than this value

5 C57/BL6 mice were immunized once or twice with varying doses of MRKAd5hCMV-nef(G2A,LLAA) (E3+), MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and(3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. The immune response were analyzed using similar protocols and the results are listed in Table 11. While anti-nef IgG responses could not be detected in this model system with any of the constructs, there are strong indications of a cellular immunity generated against nef using the ELIspot assay.

Table 11. Immunogenicity of MRKAd5nef Vectors in C57/BL6 mice.

				A	nti-nef IgG Tit	ers*	l s	FC/10^6 cell	sb
Group	Vaccine	Dose -	No. of Doses	GMT	+SE	-SE	Medium	aa51-70 CD8+	aa81-100 CD4+
1	MRKAd5hCMVFLnef (E3+)	10^7 vp	2 1	174 132	70 42	50 32	1(1) 0(0)	23(1) 0(0)	1(1) 0(0)
2	MRKAd5hCMVFLnef (E3+)	10^9 vp	2	174 132	70 42	50 32	0(0) 1(1)	61(7) 62(7)	4(2) 3(1)
3	MRKAd5mCMVFLnef (E3+)	10^7 vp	2	132 115	42 46	32 33	3(1) 3(2)	15(5) 3(2)	5(2) 4(2)
4	MRKAd5mCMVFLnef (E3+)	10^9 vp	2 1	132 132	42 42	32 32	4(2) 2(1)	83(13) 29(2)	5(1) 4(0)
5	MRKAd5mCMVtpanef(E3+)	10^7 vp	2	132 100	42 0	32	3(2) 3(1)	14(2) 13(4)	5(1) 10(3)
6	MRKAd5mCMVtpanef(E3+)	10^9 vp	2	230 115	170 46	98 33	3(2) 7(1)	145(29) 151(14)	4(0) 10(0)
7	Naïve	none	none	152	78	52 ·	21(2)	18(6)	26(3)

\*GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the gemetric mean

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Monkey Studies - Cohorts of 3 rhesus macaques were immunized with 2 doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). The number of antigen-specific T cells (per million PBMCs) were enumerated using one of two

No. of Spot-forming Cells per million spleonoytes; mean values of triplicates are reported along with standard errors in parenthesis.

No. of spot-forming cells per million splechoytes; mean values of triplicates are reported along with standard errors in parenthesis.

peptide pools (L and R) that cover the entire pol sequence; the results are listed in Table 12. Moderate-to-strong T cell responses were detected in the vaccinees using either constructs even at a low dose of 10^9 vp. Longitudinal analyses of the anti-RT antibody titers in the animals suggest that the pol transgene product is expressed efficiently to elicit a humoral response (Table 13). It would appear that generally higher immune responses were observed in animals that received the E3- construct compared to the E3+ virus.

Table 12. Pol-specific T Cell Responses in MRKAd5pol Immunized Rhesus

10 Macaques.

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Vaccine (T=0.4 wks)	Monk #		Prebleec	1		_ T=4			T=7			T=16	
		Mock	PolL	Pol R	Mock	Poi L	Pol R	Mock	Pol L	Pol R	Mock	PolL	Pol R
MRKAd5hCMV-IApod(E3+)	990100	1	0	0	1	38	31	0	52	146	٥	49	715
10^11 vp	99C215	Ιi	2	2	10	98	249	1	109	305	22	88	250
10 11 45	99D201	5	5	4	6	149	95	0	40	35	0	35	18
MRKAd5hCMV-IApol(E3+)	99D212	0	2	0	4	331	114	0	58	14	0	6	6
10'9 VD	99D180	0	4	2	0	19	192	4	38	156	5	38	106
	99C2D1	8	5	21	6	62	62	0	18	32	)	14	65
MRKAd5hCMV-IApd(E3-)	99D239	5	2	2	20	82	172	1	68	114	9	21	40
10^11 vo	99C186	4	12	6	5	120	421	2	271	489	16	875	530
, , ,	99C084	1	8	9	8	84	484	0	14	236	ו	24	264
MRKAd5hCMV-IApd(E3-)	CC7C	10	10	8	12	724	745	4	322	376	4	188	176
10/9 vp	CDIG	2	0	1	5	474	458	0	232	212	0	101	121
	CD11	6	6	12	10	98	110	5	6D	80	8	25	34
Nove	083Q	nd	nd	nd	nd	nd	nd	4	2	2	2	1	2

nd, not determined Recorded are SFC per million PBMCs: mean of dublicate wells.

Table 13. Anti-RT Ig Levels in MRKAd5pol Immunized macaques.

	T		
nL			
T =4	T=7	T=12	T=16
61	1999	5928	4768
81	1541	2356	2767
53	336	539	387
10	40	49	68
<10	36	79	93
<10	37	71	76
		<u></u>	
44	460	1234	1015
21	· 233 ·	480	345
235	2637	2858	1626
32	175	306	235_
20	140	273	419
15	112	149	237
	1 = 4 61 81 53 10 <10 <10 21 235	T=4 T=7  61 1999 81 1541 53 336  10 40 <10 36 <10 37  44 460 21 233 235 2637  32 175 20 140	T=4         T=7         T=12           61         1999         5928           81         1541         2356           53         336         539           10         40         49           <10

When rhesus macaques were immunized i.m. with two doses of MRKAd5nef constructs, vigorous T cell responses ranging from 100 to as high as 1100 per million were observed in 8 of 12 vaccinees (Table 14). The efficacies of the mCMV- and hCMV- driven nef constructs are comparable on the basis of the data generated thus far.

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Table 14. Nef-specific T cell Responses in MRKAd5nef Immunized Rhesus Macaques.

Vaccine (T=0,4 wks)	Monk #	Р	re	T	=4	T:	=7	T≘	16
		Mock	Nef	Mock	Nef	Mock	Nef	Mock	Nef
MRKAd5hCMV-nel(G2A,LLAA) (E3+)	CD2D	0	4	31	440	4	368	1	251
10^11 vp	CC7B	0	0	2	521	0	178	1	1522
· · · · · ·	CC61	2	9	31	112	0	108	11	100
MRKAd5hCMV-nef(G2A,LLAA) (E3+)	CC2K	9	9	6	52	0	35	0	15
10^9 vp	CD15	5	4	30	998	2	586	0	434
·	CD16	6	1	6	1146	0	369	1	212
MRKAd5mCMV-nef(G2A,LLAA) (E3+)	99D191	1	5	4	614	0	298	2	419
10^11 vp	99D144	4	6	5	434	0	1100	2	932
·	99C193	1	2	1	58	1	22	0	64
MRKAd5mCMV-nef(G2A,LLAA) (E3+)	99D224	1	11	14	231	1	125	0	70
10^9 VD	99D250	8	9	4	108	0	54	0	5
•	99C120	1	6	20	299	0	92	.0	79
Naîve	083Q	nd	nd	18	22	4	5	2	1

#### EXAMPLE 25

Comparison of Clade B vs. Clade C T Cell Responses in HIV-Infected Subjects PBMC samples collected from two dozens of patients infected with HIV-1 in US were tested in ELISPOT assays with peptide pools of 20-mer peptides overlapping by 10 amino acids. Four different peptide pools were tested for cross-clade recognition, and they were either derived from a clade B-based isolate (gag H-b; nef-b) or a clade C-based isolate (gag H-c, nef-c). Data in Table 15 shows that T cells from these patients presumably infected with clade B HIV-1 could recognize clade C gag and nef antigens in ELISPOT assay. Correlation analysis further demonstrated that these T cell responses against clade C gag peptide pool were about 60% of the clade B counterpart (Figure 24), while the T cell responses against clade C nef were about 85% of the clade B counterpart (Figure 25). These results suggest that cellular immune responses generated in patients infected with clade B HIV-1 can recognize gag and nef antigens derived from clade C HIV-1. These data show that a HIV vaccine, such as a DNA or MRKAd5-based adenoviral vaccine expressing a clade B

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gag and/or nef antigen will potentially have the ability to provide a prophylactic and/or therapetic advantage on a global scale.

Table 15 Responses Shown as the Number of gIFN-Secreting T Cells per Million **PBMCs** 

subject	bleed date	gag epitope #	mock	gag H-b	gagH-c	nef-b	nef-c
		(from mapping)		·			
#100	19-Jul-99	12	10	3950	1385	1295	1300
#101	25-Jul-99	3	15	3885	1280	na	1020
#102	25-Jul-99	4	15	1740	850	1255	1785
#104	7-Jun-99	2	5	1355	1185	na	1060
#107	11-Oct-99	2	25	3305	2795	670	870
#405	11-Jul-99	2	15	4575	3180	1700	1500
#501	19-Jul-99	2	15	1100	570	3365	3460
#505	18-Jul-99	5	10	2145	1725	1235	na
#506	28-Feb-99	2	25	150	45	400	610
#701	28-Mar-99	5	30	7620	4775	3320	2780
<b>#709</b>	17-May-99	3	15	2785	1945	1090	1630
#710	24-May-99		5	1055	1080	2210	2140

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## EXAMPLE 26

## Characterization and Production of MRKAd5pol and MRKAd5nef Vectors in Roller Bottles

Expansion of nef and pol Adenovectors - Nef and pol CsCl purified MRKAd5 seeds were used to infect roller bottles to produce P4 virus to be used as a seed for further experiments. P4 MRKAd5 pol and nef vectors were used to infect roller bottles at an MOI 280 vp/cell, except for hCMV-tpa-nef [E3+] which was infected at an MOI of 125 due to low titers of seed obtained at P4.

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Table 16 Viral particle concentrations for P5 nef and pol adenovectors

Adenovector	AEX Titer	AEX Titer	Amplification
	(10 <sup>10</sup> vp/ml culture)	(10 <sup>4</sup> vp/cell)	Ratio
hCMV-FL-nef [E3+]	1.1	0.9	30
mCMV-FL-nef [E3+]	2.2	2.1	75
hCMV-tpa-nef [E3+]	0.07	0.1	5
mCMV-tpa-nef [E3+]	1.3	0.9	35
hCMV-FL-pol [E3+]	2.7	2.1	75
hCMV-FL-pol [E3-]	1.9	1.3	45

5 Roller Bottle Passaging - Passaging of the pol and nef constructs continued through passage seven. Cell-associated (freeze/thaw lysis) and whole broth (tritonlysis) titers obtained in all passages were very consistent. In general, MRKAd5pol is ca. 70% as productive as MRKAd5gag while MRKAd5nef is ca. 25% as productive as MRKAd5gag. Samples of P7 virus for both constructs were analyzed by V&CB by restriction digest analysis and did not show any rearrangements.

Table 17. Passage Six Viral Productivity for MRKAd5pol and MRKAd5nef

			0° cells/ml), ity (%)   Harvest	Cell Passage Number	AEX Titer (Cell Associated) 10 <sup>10</sup> vp/ml culture	Titer  10° vp/cell	Amplification  Ratio	Triton Lysis Titer  10 <sup>10</sup> vp/ml culture
hCMV-FL-nef [B3+]	pool	1.22, 85%		62	0.8	0.7	25	1.6
	1 2		0.99, 62% 1.10, 72%					
hCMV-FL-pol [E3+]	pool	1.42, 89%		62	4.5	3.2	115	7.0
	1 2		1.22, 70% 1.42, 74%					

15 Table 18. Passage Seven Viral Productivity for MRKAd5pol and MRKAd5nef

			0 <sup>6</sup> cells/ml), ity (%)	Cell Passage	AEX Titer (Cell Associated)	Titer	Amplification	Triton Lysis Titer
		Infection	Harvest	Number	10 <sup>10</sup> vp/ml culture	10 <sup>4</sup> vp/cell	Ratio	10 <sup>10</sup> vp/ml culture
hCMV-FL-nef [E3+]	Pool	1.33, 90%		66	1.0	0.8	29	2.1
			0.96, 70%			.,		
	2		1.18,73%					
hCMV-FL-pol [B3+]	`Pool	0.90*, 90%		56	4.2	4.7	168	6.5
	1		1.18, 88%					
	2		1.04, 80%					

MRKAd5nef and MRKAd5pol Viral Production Kinetics - A timecourse experiment was carried out in roller bottles to determine if the viral production kinetics of the MRKAd5pol and MRKAd5nef vectors were similar to those of MRKAd5gag. PER C6<sup>©</sup> cells in roller bottle cultures were infected at an MOI of 280 vp/cells with P5 MRKAd5pol, P5 MRKAd5nef and P7 MRKAd5gag; for each adenovector, two infected bottles were sampled at 24, 36, 48, and 60 hours post infection. In addition, two bottles were left unsampled until 48 hpi when they were harvested under the Phase I process conditions. The anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36,

48, and 60 hpi timepoints are shown in Figure 29A-B. The QPA titers show a similar trend (data not shown).

Comparison of hCMV- and mCMV-FL-nef - As the titers obtained with the

MRKAd5nef construct (hCMV-FL-nef) were lower than those obtained with
MRKAd5gag or MRKAd5pol, a viral productivity comparison experiment was
performed with mCMV-FL-nef. For each of the two adenovectors (hCMV- and
mCMV-FL-nef), two roller bottles were infected at an MOI of 280 vp/cell with
passage five clarified lysate. The macroscopic and microscopic observations of the
four roller bottles were identical at the time of harvest. Analysis of the clarified lysate
produced indicated a higher viral particle concentration in the bottles infected with
mCMV-FL-nef, as shown in Table 19. It is stipulated that the higher productivity with
mCMV promoter driven nef vector is due to lower nef expression levels in PER.C6®
cells- experiments are underway at V&CB to measure nef expression levels.

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Table 19. Passage Six Viral Productivity Comparison of hCMV- and mCMV-FL-nef

		Xv (10 <sup>s</sup> cells/m	d). Viability (%)	Cell Passage	AEX Titer	Titer	Amplification	Triton Lysis Titer
		Infection	Harvest	Number	10 <sup>10</sup> vp/ml culture	104 vp/cell	Ratio	10 <sup>10</sup> vp/ml culture
hCMV-FL-nef	Pool	1.11, 91%		. 60	1.5	1.4	50	2.8
(MRKAd5nef)	1		1.23,75%			-		-
	2		1.34, 74%		}			
mCMV-FL-nef	Pool	1.11, 91%		60	2.3	2.1	75	4.6
	i		1.49, 84%					
	2		1.18,77%					

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#### **EXAMPLE 27**

# Characterization and Large Scale Production of MRKAd5nef Virus in Bioreactors

Materials and Methods - The experiment of the present example was run twice under the following conditions: 36.5°C, DO 30%, pH 7.30, 150rpm agitation rate, no sparging, Life Technologies (Gibco, Invitrogen) 293 SFM II (with 6mM L-glutamine), 0.5M NaOH as base for pH control. During the first run (B20010115), two 10L stirred vessel bioreactors were inoculated with PER.C6® cells at a concentration of 0.2x106 cells/ml. Cells were grown until they reached a cell concentration of approximately 1x106 cells/ml. The cells were infected with uncloned MRKAd5nef (G2A,LLAA) at a MOI of 280 virus particles (vp)/cell. For the second batch (B20010202), the same procedure as the first run was used, except the cells

were infected with cloned MRAd5nef. During both runs, the bioreactors were harvested 48 hours post-infection. Samples were taken and virus concentrations were determined from whole broth (with triton lysis), supernatant, and cell pellets (3 X freeze/thaw) with the AEX and QPA assays. Metabolites were measured with BioProfile 250 throughout the process.

Table 20: Experimental Conditions

Temperature	36.5 °C		
DO	30%	·	
PH	7.30		
Agitation	150 rpm		
Sparging	None		•

Table 21: Virus source used for experiments.

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Run	Batch ID	Cloned/Uncloned	MOI
ł		MRKAd5nef	(vp/cells)
#1	B20010115-1	Uncloned	280
	B20010115-2	Uncloned	280
#2	B20010202-1	Cloned	280
	B20010202-2	Cloned	280

Results - Table 22 and 23 show an the ability to scale up production of MRKAd5nef by growth in a bioreactor.

Table 22: Virus Concentration as measured by the AEX assay 15

Run	Batch ID	Cloned/Uncloned	Virus Concentration @ 48hpi (1x10 <sup>13</sup> vp/L)						
		MRKAd5nef	Supernatant	Clarified Lysate	Total	Triton Lysate			
#1	B20010115-1	Uncloned	0.72	3.26	3.98	5.76			
	B20010115-2	Uncloned	0.38	1.67	2.05	2.46			
#2	B20010202-1	Cloned	0.80	6.00	6.80	8.88			
	B20010202-2	Cloned	0.50	6.00	6.50	8.47			

Table 23: Virus Titers as measured by the QPA assay

Run	Batch ID	Cloned/Uncloned	Virus Concentration @ 48hpi (1x10 <sup>11</sup> IU/L)					
		MRKAd5nef	Whole Broth	Supernatant	Clarified Lysate	Total	Triton Lysate	
#1	B20010115-1	Uncloned	0.13	1.12	1.76	2.88	11.28	
	B20010115-2	Uncloned	0.14	0.73	1.54	2.27	5.86	
#2	B20010202-1	Cloned	0.14	0.97	1.62	2.69	11.89	
1	B20010202-2	Cloned	0.14	1.17	1.70	2.97	12.47	

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The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art

from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

## EXAMPLE 28

MRKAd5HIV-1gag Boosting of DNA-Primed Animals

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Groups of 3-5 rhesus macaques were immunized with (a) 5 mgs of V1Jns-Flgag (pVIJnsCMV(no intron)-FL-gag-bGHpA), (b) 5 mgs of V1Jns-Flgag formulated with 45 mgs of a non-ionic block copolymer CRL1005, or (c) 5 mgs of V1Jns-Flgag formulated with 7.5 mgs of CRL1005 and 0.6 mM benzalkonium chloride at weeks 0, 4, and 8. All animals received a single dose of 10e7 viral particles (vp) of the MRKAd5HIV-1gag at week 26. Note: 10e7 is too low to prime or boost effectively when used as a single modality (dose is selected to mimic preexposure to adenovirus); see Figure 32.

Blood samples were collected from all animals at several time points and peripheral blood mononuclear cells (PBMCs) were prepared using standard Ficoll method. The PBMCs were counted and analyzed for gamma-interferon secretion using the ELISpot assay (Table 24). For each monkey, the PBMCs were incubated overnight either in the absence (medium) or presence of a pool (called "gag H") of 50 20-aa long peptides that encompass the entire HIV-1 gag sequence.

The results indicate that MRKAd5HIV-1gag was very effective in boosting the T cell immune responses in these monkeys. At week 28 or 2 weeks after the viral boost, the number of gag-specific T cells per million PBMCs increased 2-48 fold compared to the levels observed at week 24 or 2 weeks prior to the boost.

The PBMCs were also analyzed by intracellular gamma-interferon staining prior to (at week 10) and after the MRKAd5gag boost (at week 30). The results for select animals are shown on Figure 31. The results indicate that (a) immunization with DNA/adjuvant formulation elicited T cell responses which can either be balanced, CD4<sup>+</sup>-biased or CD8<sup>+</sup>-biased, and (b) boosting with the MRKAd5gag construct produced in all cases a strongly CD8<sup>+</sup>-biased response. These results suggest that boosting with MRKAd5HIV-1gag construct is able to improve the levels of antigen-specific CD8<sup>+</sup> T cells.

385 385 959 1916 836 1549 1229 25 88 82 872 273 88 88 872 28 38 88 88 322 88 32 8 85886 989 H 224 58 46 270 164 530 530 530 8 2 2 2 8 284 1288 118 28 th 28 th 28 th 82558 8 1 2 a 8 15 00\$2 | Table 24. Boosting of DNA/Adjuvant-Primed Rhesus Monkeys with MRKAd5gag
Number of SFC/million PBMCs	Boost	Monke	Table wis	Nedium	
Table 4,8 wks	Table wis	Nedium			
DNA/6 mgs	MRKAd5gag(E3+)	CBSH	NA		
DNA/6 mgs	OFF	OFF	OFF	OFF	OFF
(D101)	AW3G	5 0 4 0 × 0 AW20 CA4R CB5W CB5W CB7D CC1C CC1K AW3P CB6F AKBB MFIKAd5gag(E3+) 10v7 vp MPKAd5gap(E3+) 10\*7 vp DNA/5 mgs+ CRL1005/7.5 mgs + 0.6 mM BAK DN/V6mgs + CRL1005/45mgs 4 NA, not available			

### **EXAMPLE 29**

# Construction of gagpol fusion for MRKAd5gagpol fusion constructs

The open reading frames for the codon-optimized HIV-1 gag gene was fused directly to the open reading frame of the IA pol gene (consisting of RT, RNAseH and integrase domains) by stepwise PCR. Because the gene (SEQ ID NO: 38) does not include the protease gene and the frameshift sequence, it encodes a single polypeptide of the combined size of p55, RT, RNAse H and integrase (1350 amino acids; SEQ ID NO: 39).

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The fragment that extends from the BstEII site within the gag gene to the last non-stop codon was ligated via PCR to a fragment that extends from the start codon of the IApol to a unique BamHI site. This fragment was digested with BstEII and BamHI. Construction of gag-IApol fusion was achieved via three-fragment ligation involving the PstI-BstEII gag digestion fragment, the BstEII/BamHI digested PCR product and long PstI/BamHI V1R-FLpol backbone fragment.

The MRKAd5-gagpol adenovirus vector was constructed using the BglII fragment of the V1R-gagpol containing the entire ORF of gag-IApol fusion gene.

### **EXAMPLE 30**

Immunogenicity Studies in Non-Human Primates

Cohorts of three (3) macaques were immunized with 10e8 or 10e10 viral particles (vp) of one of the following MRKAd5 HIV-1 vaccines: (1) MRKAd5gag; (2) MRKAd5pol; (3) MRKAd5nef; (4) a mixture containing equal amounts of MRKAd5gag, MRKAd5pol, and MRKAd5nef, or (5) a mixture of equal amounts of MRKAd5gagpol and MRKAd5nef. The vaccines were administered at weeks 0 and 4.

The T cell responses against each of the HIV-1 antigens were assayed by IFN-gamma ELISpot assay using pools of 20-aa peptides that encompass the entire protein sequence of each antigen. The results (Table 25) are expressed as the number of spot-forming cells (sfc) per million peripheral blood mononuclear cells (PBMC) that respond to each of the peptide pools.

Results indicate the following observations: (1) each of the single gene constructs (MRKAd5gag, MRKAd5pol, or MRKAd5nef) is able to elicit high levels of antigen-specific T cells in monkeys; (2) the single-gene MRKAd5 constructs can be mixed as a multi-cocktail formulation capable of eliciting very broad T cell responses against gag, pol, and nef; (3) the MRKAd5 vector expressing the fusion

protein of gag plus IA pol is capable of inducing strong T cell responses to both gag and pol.

Table 25. Evaluation of Mixtures of MRKAd5 vectors expressing humanized

HIV-1 gag, pol, gagpol, nef in rhesus macaques

Grp#	Vaccine	Monk #			T=6 wks		
	T=0, 4 wks		Mock	Gag H	Pol - 1	Pol-2	Net
1	MRKAd5 gag	CB9V	0	15		-	•
- 1	10^10 vp	CD19	0 .	374	-	-	-
		109H	1	843	-	-	-
2	MRKAd5 gag	99D130	1	948	-	-	-
l	10^8 vp	W277	16	324		•	-
		143H	4	595	-	- '	•
3	MRKAd5 pol	CC1X	4	-	46	256	-
I	10^10 vp	AW3W	3	-	463	550	-
		AV43	6	- /	95	1333	•
4	MRKAd5 pol	AW38	1	•	19	30	-
- 1	10^8 vp	CC8K	0	-	50	995	-
		CC21	1	-	33	436	-
5	MRKAd5 nef	076Q	9	-	-		120
	10^10 vp	091Q	4	-	-	- [	85
		083Q	0	-	-	-	170
6	MRKAd5 nef	00C029	1	-	_	_	114
- 1	10^8 vp	98D022	6	-	-	- 1	17
		98D160	3	-	-	-	19
7	MRKAd5gag+MRKAd5pol+MRKAd5nef	99D251	3	206	15	193	120
	10^10 vp each	05H	3	135	21	9	638
		00C016	3	26	4	51	23
8	MRKAd5gag+MRKAd5pol+MRKAd5nef	99D215	7	171	18	193	24
	10^8 vp each	81H	5	73	6	14	24
		12H	8	1140	115	811	719
9	MRKAd5gagpol +MRKAd5 nef	99D211	0	83	56	838	72
}	10^10 vp each	22H	4	385	119	1194	191
		61H	4	343	11	765	853
10.	MRKAd5gagpol +MRKAd5 nef	34H	3	78	19	5	75
ļ	10^8 vp each	48H	1	65	105	46	43
		70H	5	158	15	220	191

Indicated are numbers of spot-forming cells per million PBMCS against the peptide pools. Mock, no peptides; gag H, fifty 20-aa peptides encompassing p55 sequence; pol-1, 20-aa peptides representing N-terminal half of IA pol; pol-2, 20-aa peptides representing the carboxy-terminal half of IA pol; nef, 20-aa peptides encompassing the entire wild-type nef sequence. Responses to the antigens prior to the first immunization did not exceed 40 sfc/10^6 PBMC.

## WHAT IS CLAIMED IS

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A recombinant adenoviral vaccine vector at least partially deleted in
 E1 and devoid of E1 activity, comprising:

- a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to between from about base pair 400 to about base pair 458 of a wildtype adenovirus genome; and
- b) a gene encoding an HIV protein or immunologically relevant modification thereof.
- A vector in accordance with claim 1 comprising a packaging region corresponding to from about base pair 1 to about base pair 450 of a wildtype adenovirus genome.
- 3. A vector in accordance with claim 1 further comprising nucleotides corresponding to between from about base pair 3511 to about 3524 to about base pair 5798 of a wildtype adenovirus genome.
  - A vector in accordance with claim 3 comprising base pairs corresponding to 1-450 and 3511-5798 of a wildtype adenovirus genome.
- 5. A vector in accordance with claim 4 which is deleted of base pairs451-3510.
  - 6. A vector in accordance with claim 1 which is at least partially deleted in E3.
  - 7. A vector in accordance with claim 6 wherein the E3 deleted region is from base pairs 28,133-30,818.

8. A vector in accordance with claim 1 wherein the gene encoding the HIV protein or modification thereof comprises codons optimized for expression in a human.

- 9. A vector in accordance with claim 1 wherein the vector comprises a gene expression cassette comprising:
  - a) a nucleic acid encoding a protein;

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- b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and
  - (c) a transcription termination sequence.
- 10 10. A vector in accordance with claim 9 wherein the gene expression cassette is inserted into the E1 region.
  - 11. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 parallel orientation
  - 12. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 antiparallel orientation.
    - 13. An adenoviral vector in accordance with claim 9 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
    - 14. An adenoviral vector in accordance with claim 13 wherein the promoter is an immediate early human cytomegalovirus promoter.
- 20 15. An adenoviral vector in accordance with claim 9 wherein the promoter is a murine cytomegalovirus promoter.
  - 16. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

17. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a synthetic polyadenylation signal (SPA).

- 18. A cell comprising the adenoviral vector of claim 1.
- 19. Recombinant, replication-defective adenovirus particles harvested
   and purified subsequent to transfection of the adenoviral vector of claim 1 into a cell
   line which expresses adenovirus E1 protein at complementing levels.
  - 20. An HIV vaccine composition comprising purified adenovirus particles of claim 19.
- 21. An HIV vaccine composition of claim 20 which comprises aphysiologically acceptable carrier.
  - 22. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 1 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
  - 23. A method according to claim 22 wherein the cell is a PER.C6® cell.

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- 24. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.
  - 25. A method according to claim 24 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

26. A method according to claim 25 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

- 27. A method according to claim 24 wherein the adenovirus vaccine is
   5 preceded by an adenovirus vaccine of a different serotype.
  - 28. A method according to claim 24 which comprises administering and readministering the adenovirus vaccine vector to the individual.
  - 29. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV gag or an immunologically relevant modification thereof.
- 30. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV gag protein or immunologically relevant modification thereof.
  - 31. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

b) a gene expression cassette comprising

- i) SEQ ID NO: 29;
- ii) a heterologous promoter operatively linked to i); and
- iii) a transcription termination sequence.

32. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 parallel orientation.

- 33 An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 antiparallel orientation.
- 34. An adenoviral vector in accordance with claim 31 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

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- 35. An adenoviral vector in accordance with claim 31 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 36. An adenoviral vector in accordance with claim 31 which is at least partially deleted in E3.
  - 37. A cell comprising the adenoviral vector of claim 30.
  - 38. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 30 into a cell line which expresses adenovirus E1 protein at complementing levels.
  - 39. An HTV vaccine composition comprising purified adenovirus particles of claim 38.
  - 40. An HIV vaccine composition of claim 39 which comprises a physiologically acceptable carrier.
- 20 41. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 30 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

42. A method according to claim 41 wherein the cell is a PER.C6® cell.

43. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.

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- 44. A method according to claim 43 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
- 45. A method according to claim 44 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.
  - 46. A method according to claim 43 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.
  - 47. A method according to claim 43 which comprises administering and readministering the adenovirus vaccine vector to the individual.
    - 48. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV pol or an immunologically relevant modification thereof.
- 49. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV pol protein or immunologically relevant modification thereof.
  - 50. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

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- b) a gene expression cassette comprising
  - a nucleotide sequence selected the group consisting of SEQ ID NO: 1, SEQ ID NO: 5 and SEQ ID NO: 7;
  - ii) a heterologous promoter operatively linked to i); and
  - iii) a transcription termination sequence.

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- 51. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 parallel orientation.
- 52. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 antiparallel orientation.
- 53. An adenoviral vector in accordance with claim 50 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
- 54. An adenoviral vector in accordance with claim 50 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 55. An adenoviral vector in accordance with claim 50 which is at least partially deleted in E3.
  - 56. A cell comprising the adenoviral vector of claim 49.

57. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 49 into a cell line which expresses adenovirus E1 protein at complementing levels.

58. An HIV vaccine composition comprising purified adenovirus particles of claim 57.

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- 59. An HIV vaccine composition of claim 58 which comprises a physiologically acceptable carrier.
- 60. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 49 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
- 61. A method according to claim 60 wherein the cell is a PER.C6® cell.
- 62. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 59.
  - 63. A method according to claim 62 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
  - 64. A method according to claim 63 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

65. A method according to claim 62 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

- 66. A method according to claim 62 which comprises administering and readministering the adenovirus vaccine vector to the individual.
- 67. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV nef or an immunologically relevant modification thereof.

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- 68. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV nef protein or immunologically relevant modification thereof.
- 69. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:
  - a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
  - b) a gene expression cassette comprising
    - a nucleotide sequence selected the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 and SEQ ID NO: 15;
    - ii) a heterologous promoter operatively linked to i); and
    - iii) a transcription termination sequence.
- 70. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 parallel orientation.

71. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 antiparallel orientation.

- 72. An adenoviral vector in accordance with claim 69 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
- 73. An adenoviral vector in accordance with claim 69 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

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- 74. An adenoviral vector in accordance with claim 69 which is at least partially deleted in E3.
  - 75. A cell comprising the adenoviral vector of claim 68.
- 76. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 68 into a cell line which expresses adenovirus E1 protein at complementing levels.
- 77. An HIV vaccine composition comprising purified adenovirus particles of claim 76.
  - 78. An HIV vaccine composition of claim 77 which comprises a physiologically acceptable carrier.
  - 79. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 68 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
  - 80. A method according to claim 79 wherein the cell is a PER.C6<sup>®</sup> cell.

81. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 78.

- 82. A method according to claim 81 which further comprises

  administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
  - 83. A method according to claim 82 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

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- 84. A method according to claim 81 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.
- 85. A method according to claim 81 which comprises administering and readministering the adenovirus vaccine vector to the individual.
- 86. A multivalent adenovirus vaccine composition comprising recombinant, replication-defective adenovirus particles, wherein the adenovirus particles are harvested and purified from a cell line expressing adenovirus E1 protein, and wherein the particles are harvested subsequent to transfection of the cells with an adenoviral vector or vectors in accordance with claim 9; said vector(s) comprising a gene expression cassette or cassettes comprising nucleotide sequences encoding HIV proteins selected from the group consisting of:
  - a) gag, pol, and nef, expressed independently from three individual vectors;

 b) gag, pol, and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;

- c) gag, pol, and nef, expressed via two vectors, one expressing a polnef fusion, and another expressing gag;
- d) gag, pol, and nef, expressed via two vectors, one expressing a gagpol fusion and another expressing nef;
- e) gag, pol and nef, expressed via two vectors, one expressing a nefgag fusion and another expressing pol;
- f) gag, pol, and nef, expressed via one vector expressing a gag-polnef fusion;
- g) gag and pol, expressed independently from two individual vectors;
- h) gag and pol, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- i) pol and nef, expressed independently from two individual vectors;
- j) pol and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- k) nef and gag, expressed independently from two individual vectors;
- nef and gag, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- m) gag and pol, expressed via one vector expressing a gag-pol fusion;

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n) pol and nef, expressed via one vector expressing a pol-nef fusion; and

- o) nef and gag, expressed via one vector expressing a nef-gag fusion.
- 87. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the gag-pol fusion consists of SEQ ID NO: 39.
  - 88. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the fused sequences have the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences.
- 89. A multivalent adenovirus vaccine composition in accordance with

  10 claim 86 wherein the fused sequences have the encoding nucleic acid sequences

  operatively linked to a single promoter; and the encoding nucleic acid sequences

  operatively linked by an internal ribosome entry sequence ("IRES").

# Original Adenovector Construct:

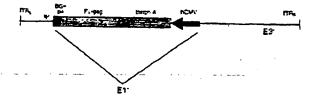


Figure 1: Original HIV-1 gag adenovector.

# Sequence of the open reading frame for FL-gag (human codon optimized)

atgggtgctagggcttctgtgctgtctggtggtgagctggacaagtgggagaagatcaggctgaggcctggtgg caagaagaagtacaagctaaagcacattgtgtgggcctccagggagctggagaggtttgctgtgaaccctggc agctgaggtccctgtacaacacagtggctaccctgtactgtgtgcaccagaagattgatgtgaaggacaccaag gaggecetggagaagattgaggaggagcagaacaagtecaagaagaaggeceageaggetgetgetgee acaggcaactccagccaggtgtcccagaactaccccattgtgcagaacctccagggccagatggtgcaccag gccatctcccccggaccctgaatgcctgggtgaaggtggaggagaaggccttctcccctgaggtgatccc catglicitgcctgtctgagggtgccacccccaggacctgaacaccatgctgaacacagtggggggccatc aggetgecatgeagatgetgaaggagaceateaatgaggaggetgetgagtgggacaggetgeateetgtge acgetggececattgececeggecagatgagggageceaggggetetgaeattgetggeaecacetecacect ccaggagcagattggctggatgaccaaccaccccccatccctgtgggggaaatctacaagaggtggatcat ccigggccigaacaagatig:gaggatgtactcccccacciccatcciggacatcaggcagggccccaaggag cccticagggactatgtggacaggttctacaagaccctgagggctgagcaggcctcccaggaggtgaagaact ggatgacagagaccctgctggtgcagaatgccaaccctgactgcaagaccatcctgaaggccctgggccctg gctgaggccatgtcccaggtgaccaactccgccaccatcatgatgcagagggcaacttcaggaaccagag gaagacagtgaagtgcttcaactgtggcaaggtgggccacattgccaagaactgtagggcccccaggaaga agggctgctgggaagtgtggcaaggaggccaccagatgaaggactgcaatgagaggccaggccaacttcctg ggcaaaatctggccctcccacaagggcaggcctggcaacttcctccagtccaggcctgagcccacagcccct agctglacccctggcctccctgaggtccctgtttggcaacgacccctcctcccagtaaaataaagcccgggca gat (SEQ ID NO: 29)

Figure 2

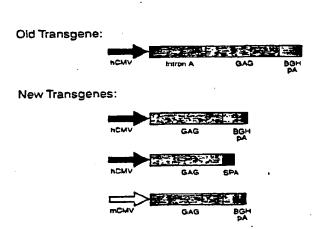


Figure 3: Diagrammatic representation of the original HIV-1 gag transgene and the series of new transgene constructions.

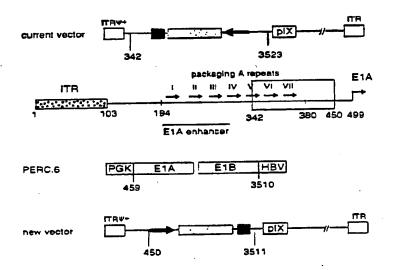


Figure 4: Modifications made to the current adenovector backbone in the generation of the new vector.

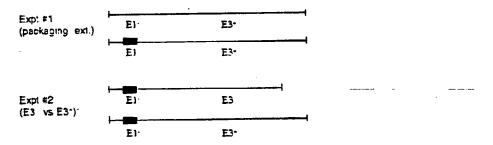


Figure 5: Virus mixing experiments to determine the effects of the addition made to the packaging signal region (Expt #1) and analysis of the effects of the E3 gene on viral growth (Expt. #2). The red bars denote the region of modifications made to the E1 deletion.

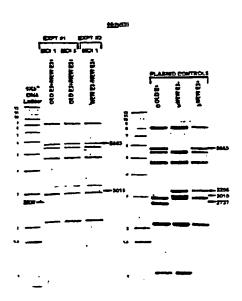


Figure 6: Autoradiograph of viral DNA analysis following viral mixing experiments (expts. #1 and #2) as detailed in the text.

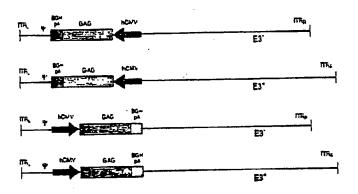


Figure 7A: hCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

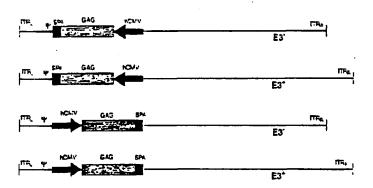


Figure 7B: hCMV-FLgag-SPA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

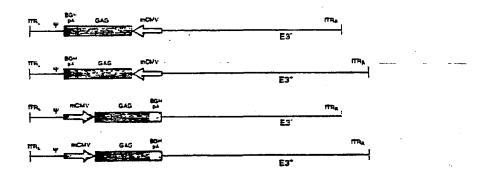


Figure 7C: mCMV-FLgag-bGHpA adenovectors constructed within the \*MRK\* backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

# Plasmid mixing expt: (orientation)

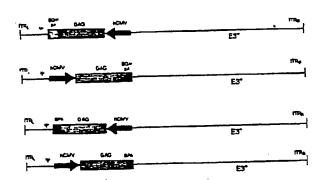


Figure 8A: Effect of transgene orientation

# Plasmid Mixing expt: (poly A signal)

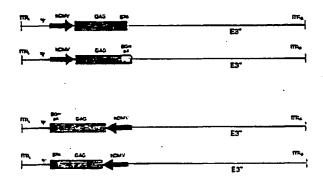


Figure 8B: Effect of polyadenylation signal

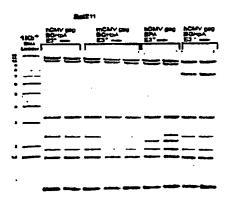


Figure 9: Viral DNA from the four Adgag candidates at P5, following BstE11 digestion.

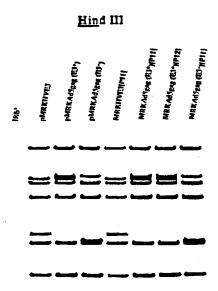


Figure 10: Viral DNA analysis of passage 11 and/or 12 of MRKHVE3, MRKAd5gag and MRKAd5gag(E3-).

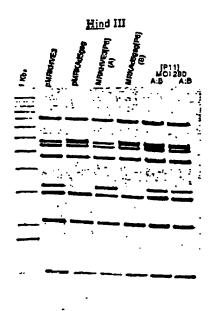


Figure 11: Viral DNA analysis (*Hin*dIII digestion) of passage 6 MRKHVE3 and MRKAd5gag used to initiate the viral competition study. Last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI 280 vp).

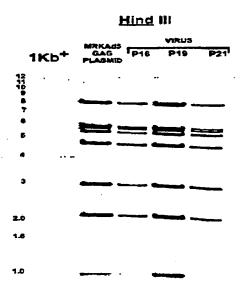
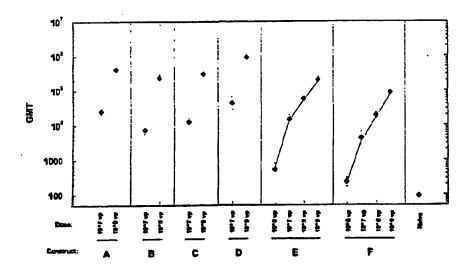


Figure 12: Viral DNA analysis by *HindIII* digestion on high passage numbers for MRKAd5gag in serum containing media with collections made at specified times. The first lane shows the 1 Kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *HindIII*), and MRKAd5gag virus continually passaged to P16, P19 and P21(serum containing media).

Figure . Serum anti-p24 Levels at 3 Wks post i.m. immunization of balb'c mice (n=10) with Varying Doses of Several Adgag constructs: (A) MRKAd5gag (through passage 5): (B) MRKAd5 E3\* hCMV-FLgag-bGHpA; (C) MRKAd5 E3\* hCMV-FLgag-SPA; (D) MRKAd5 E3\* mCMV-FLgag-bGHpA; (D) research Lot (293 cell-derived) of Ad5HIV-1gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1gag. Reported are the geometric mean titers (GMT) for each cohort.



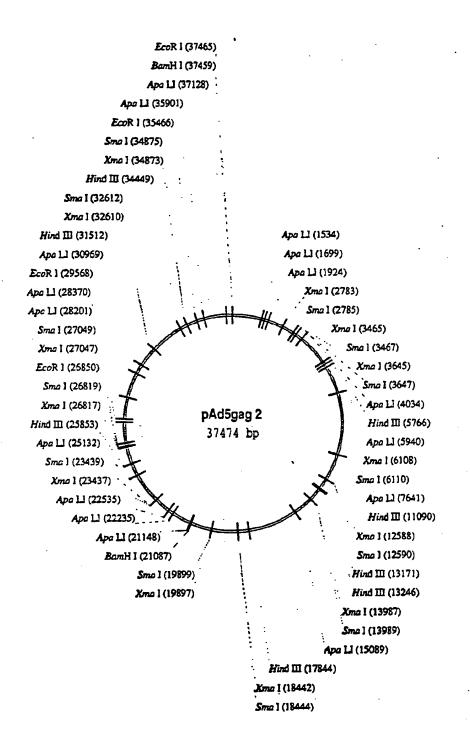


Figure 14

ACCAGAN: AA TOCTIGATION ACCACACACAC GCTKGGAGAAGG CACCTGAGGT TTACTOCATA TCATTOTAGA" CCATCCGCAC CACACCCCCC CTGTGGCCC CGACCACTACC CCTATALTAI CATTAGITICA GTENTCARGE ANTGACCTAT ATAGTATACO NGTACATOTA GGTAN CGT PATCATATY CICTOMOCO ACAGGTRAGG TCTCCACCC GACTCACGO DATTTCCAAO CTCCATAGAA AGGGCTTCTG TCCCGAAGAC ATTICAGGAGG ACCTCCAGGG TAATGCCCCA GCCCAACGAC CCCCCCCAT TGACGTCAAT ACATCAAGTG TGTAGTTCAC CTANAGGTTC GCANATGGGC CGTTTACCCG CCTCCAGGGA GGAGGTCCCT AGGCTCTGAG TCCGAGACTC TAACTCCTCC ACTOCAGTTA CCTACTTOGC GGATGAACCG GACAAACTO GAGGTATCTT CULTINCOID GCANATGCAC TACCHITCHAT ATCCAACATA ATACTAATCA ATTACGGGGT COCCUCOCOCC ححودعودحو TOACOTTETE ACTOCANANA **GCCATTITICO** COGTANAAGC CCTOGAGAAG CCCCATTGAC CHGTTTTTCAC GTACCCACGA AFTIGRETICOG TAACACACCC CCCTCCAAAC COGACOTTE **GCACCTICTTC** ATTIGTECAGA GOOGTANCTG CATGGGTGCT TAACACGTCT ACTIVENTIA TACTATTACT CCCCACCTC AAACACTGCA ATCCATIGCA TATCATTAGE ATGGGACTET THYGICACGE GTGGCNANG CACCGITITIC GTAAGATTTG CATTCTAMAC GGACTTTRGAC CCTCANACTO TAGGTAACGT COCCCCCTA ACTITICOCAGE TGAACCGTCA TACCCTIGNA CTCMCTGCCC GANCTACCCC ATCCCATATCG ATACCCCTTT AACAACTCCG ACCITATAGAC OCCATECACG CCAGATATAT TCGTCTCGAG CAAATCACTT GGCAGTCTAG CGGACCTCTG CGGTAGGTGC CATTGOANCE COGNITICECE GINECIARGAG TRAGATETAE CACCATTICTC ACTETAGATE GETTAGA AGAAGTACAA GCTAAAGCAC CGATTTCGTG GCAGGCAGAT CCTGGGCCAG CTCCAGCCCT GAGCTCGGGA CCANGGAGGC GGTTCCTCCG CTTCATCOGG GTATTTACKS TANACTIGCCC ACATGACCTT TICATTATICA CTAGITATIA TCTACTCCAA THGTHGAGGC TCACACCOCC TIGITATAT TCACTGCCTA CCAMATCCG CCTACACAT CATTFAAACC CCCATFGGCT Tecesses 2020022002 GATCAATAAT CGGGTTGCTG ATTTGACOGG TACCCCACC TATCCCCAAA ACCERACTION CHATTANCOA AGRICCCARGG Ē CATANATISC ARTICICCA TYTHICATION CHINCCCCCCTC GTT:AMTGACA CACTFICCTIGT TCCACAGGGT TATGCCCAGT ATACCCCTTCA AAAATGTCGT TITINCAGCA ATCATAATKIA CITAMATITICG TUTCHTACTC ATACACCICTA ATATITICICT TATAMACAGA TATTATTA **ATMTANTAT** ACTAATAACT CTRICCTRIACE GACCGACTOS AACACATGTA CHETICACCA GAAGATTGAT TORCACARRIC AAPTITICARRIC TICCGGGTCG TCCGACGACG ACCGTGTCCG TT\*:ACX:TCGG CCCTANGRIGG CCACCGTTCT CTICTARCTA CATTACCGCC ATCITICACAT GGGACTITICC GTTTTAGTTG CCCTGAAAGG CCGTCAGATC COTITITION GRANGINGIN TATICACION THICKGOIT CCORGICAAA GITKACGITTI CANCIGICAM TACAACTGTA ANTERCRICAC TTACCGGGCG CATTGACGITE AATGGGTGGA TTACCCACCT CCCCTTCCCAT GCCGACCCTA CAGTACATCA GTCATGTAGT CGTCCGTCTA ACTIVITY N. N. N. N. AGACTCCCCA GTGTCACCGA TGGGACATGA CACACGTCGT ACTIACGGTA CITANCCITICS CGACTCCGGA ACACANTGAG TCAATGCCAT **OTAACT**GCAG CTAAATCCCC CATTITACCOS CCCCTTTTCC COCCAMANCO CAMATCANC GTTTAGICAN GCTGAGGGCT TETEAGGGCT ATAAAACCTA TOATGETINGA ACTACAACGT ANACASCICIA GOCCCAGTIT GTAATGGCGG TATTTICKGAT TCCCTGAAAG CAGTTACTGC GGTACCACTA CAAAACCGTG ACCAGAGCTC GOGMACAGITA CCCTTGCCAC AGAAGATCAG TCTTCTAGEC CGACCTCTGG ACCURATA AGGCTRICTED GCGTTACATA ACCORPTITE GTCAATCACG **CCATCCTCAT** GTTTTGGCAC **OCTOGAGACC** COCMATGTAT TAATATATT ATTATATATA GGCGTAACTC CCCCCTTCAC TAMARCOSC TGANTANTIT ACTITATION TCATGTCCAA AGTACAGGTT ATTTICIBLE MODECCAGE AATATAACCG TACCTCAAGG TAACOCCAAT ATTOCOGITA CCCTATTGAC **GGGATAACTG** ATCGCTATTA TACCGATAAT ATCCCCACTT TACCCTCAAA GETCTATATA CHCCCCCCCC GAGGCGCCCGG GACAAGTGGG CTGTTCACCC Accetooccf TOCCACCOCA CACAGTGGCT AGAGTCCACA TTATATTGGC ATCGAGTTCC COMMUTACACA AAGTGAAATC Tereaggigt TTCTTAATTA ACATCATCAA TUTACTACT GTAGTAGTGT CATCATCACA CCTTCACTGT TTCACTITAG CAAGGGTATC GTTCATGCGG MOCCACCCT GOGACATGTT GTCCAAGAAG CAGGITTCTTC ATCCCCTATA STICCCATAG COTATTAGT CCATANTICAG NTTGACGTCA TAACTGCAGT TACCOCTOCOCA CCGATCCAGC COCTAGGTCG NOTICABOTO ACCACTCGAC PTROCTGTGA MANCGACACT CCCTGTACAA AACAATTAAT CACCOCCTOAC CCCCCCACTG CHUTACACA GANTAAGAGG **PATACATOTA** PAGCCCATAT CAAGTACGCC CCACATGROT CITAINCICC CAGGIGITIT GTCCACAAAA ATATGTACAT 1101 1301 1501 1601 1201 1401 701 801 901 1001 401 501 601 201 301 101

Figure ISA

1701	CACCAGGCCA				ActimizativasA			AGGTGATCCC	CAMITTOTOT	GCCCTGTCTG
	Greenceer		-	_	TT. M.C.M. 1. T					COMPACTOR C
1801	AGGGTGCCAC TCCCACGGTG	CCCCCARGAC	CHCANCACCA	ACCIACTIVITY	Militario and	CHINCTCCCAC	CCARCOTCTA	CGACTTCCTC	TOGTAGTTAC	TCC:TCC:AC:
1901	TGAGTGGGAC	AGGCTGCATC	CHOSTICALISC	TOGGCCCATT ACCGCGCTAA	מסטעמטעטטט מסטעמטעטטט	ACATICACOCA	הנימטטטטטט בנימטטטבטטט	TCTCACATTG AGACTGTAAC	CTOCCACCAC	CTCCMCCT++
2001	CAGGAGCAGA	-			CHUTTERIGORIA	AATCTACAAG	ACCACCTAGE TCCACCTAGE	TCCTGGGCCT AGGACCCGGA	GAACAAGATT	GTCACCTACA
2101			_		CETCCCCTTC	AGGACTATG	TGGACAGGTT	CTACAAGACC	CTGAGGGCTG	AGCANDCCTV TCGTCCGGA1
2201	CCAGGAGGTG		TGACAGAGAC	CCTGCTGGTG	CAGAATGCCA	ACCCTGACTG TGGGACTGAC	CAAGACCATC	CTGAAGGCCC	TGGGCCCTGC ACCCGGGACG	TOCCALCCTO ACGCTGGGAI
2301	CTCCTCTACT	TOACAGCCTO	CCACCCCCAC	GRAGOCCCITA	CACTECTACCG	CAGGGTGCTG	GCTGAGGCCA CGACTCCGGT	TOTCCCAGGT	GACCAACTCC	GCCACCATC, CGCTGGTAG!
2401	TGATOCAGAG ACTACGTCTC	GCCGTTGAAG	AGGAACCAGA TCCTTGGTCT	GGAAGACAGT	CTTCACGAAG	AACTGTGGCA TTGACACCGT	AGOTGGGCCA TCCACCCGGT	CATTGCCAAG GTAACGGTTC	AACTGTAGGG TTGACATCCC	CCCCCAMAUAA. GGCACATCCT !
2501	GAAGGGCTGC	TOGANGTOTO	GCANGGAGG	CCACCAGATG	MOUNTECA	ATGAGAGGCA	CCGCTTGAG	CTOCCCANAA	TCTGGCCCTC AGACCGGGAG	CCACAAGOGI: GGTGTTCCCY:
2601	AGGCCTGGCA	ACTTCCTCCA TGAAGGAGGT	GTCCAGGCCT	CTCGGGTGTC	CCCCTCCCGA	CCTCAGGAAG	AGGTTTCCAG	ACCICITICIO	CACCCCAGC	CACANOCAR!
					-					Bytil
2701	AGCCCATTGA	CAAGGAGCTG	TACCCCT00	CCTCCCTGAG	CACCCTCTTT	GREANMANCE	CCTCCTCCCA	CATTITIATE	CGGGCCCGTC	ATCTOCTOTO:
2801	CCTTCTAGTT					GACCCTGGAA	GGTGCCACTC	CCACTOTCCT	TICCTAATAA	ANTCACCAN
2901	THACARCACA				CHCCHACUCIAC				GACAATAGCA	Sphi wrwwww GCCATCCTCG
}	AACGTAGCGT	_	_	GATAAGACCC	CCCACCCCAC	cccGrccrGf	cerreceeer	CCTAACCCTT	CTGTTATCGT	CCCTÁCGACC
1				Ascl						
3001	GCTACGCCAC	GOCTCTATOG		CCGATCGGCG CCCCGTACTG GCCTAGCCGC GCCCCATGAC	AAATCHCHA:	COCACCGAAT	TCCCACCCTT	TCTTATATAT	TCCACCCCCA Sphi	GAATACATCA
3101	THIGHATCH	3 TTTTGCAGCA C AAAACGTCGT	י פככיניכנים	CCATGAGGAG	CAAÇTICITITE GETICAGUAAA	GATGGAAGGA CTAGGTTCGT	THEMS ACTORNO	ATATTTCACA TATAAACTGT	ACCICCIANCE TGCGCGTACG	CCCCATOCCC
3201	CGCGCACGCA	r cagaatetea A gevetacace	ACCERAGETE	CATTGATTAGT : GTAACTACCA	מנטששטעעט מנטששטעעט	TCCCCCCAAA ACGCCCCTTT	CTCTACTACC	TTGACCTACG AACTGGATGC	AGACCCACAG TCTCCCACAG	TOGANGGCG ACCTTGCGGC

tique 15e

Pell

101	THE STANSACTICS	CAGCCHCCGC		כשבטגוכונות שונית וויישט בראינייניני	בראוניהיינים	CONTRACTO ACTION CTTTE	ACTIVIACTEPRS (	CHITCHIGAG (	CHITCHISAG CCCGCTTGCA AACACITGCAG	<b>ANCACHTECAG</b>	
		gregoyagea		CCXCCCCACACTEC: 6		נאנינירויאונאני	TGACTGAAAC (	CHANCINCTC	GCCCCAACGT	THEFT	
401		ATCCCCCCC	CATCACAGE	TCACCECATCT	TITIVARICALIA	TYXXATTCTF !				ACKTACITY	
	GANCICICANG		CTACTOTACA	ACTRICCGAGA	AAACCCITCT.	ANCETIVICAN !				TCGACAACI "I'	
501	TCTCCCCAO	CACACITICAG	בכבוגיאאינינ	TRUTCHE	CCCAATTACK	TTTAMARTAT !	MATAAAAA (			GATCAAGCAA	
: :	AGACGCGGTC	GTCCANAGAC		AAGGAAGAGA	GEGTTACKE	AAATTTWAA	TTTATTTT (			CTAGTTCC: I'	
601	GROTETRACT	GICTITATIT	AGGGGTTTTG	רושנוצכופרומחד	אממכבבנגאטע <b>י</b>	CCASCATICT (	CONTROTTER			AGTACGTART	
• ;	CACAGAACGA			<b>GCTGCGCCCCA</b>	TCCGGGCCCT	CACTURE CAGA	۲	CCCAGGACAC	NTARARAGG	TCCTGCACCA	
							Fig.1				
101	AAACCTCACT	CTOCATOTIC		AGATACATEG CCATAAGGC GTCTCTGAGG TXXAGGTAGC	Greneronos		ACCACTRICAG AGCTACATGC	ADCTFICATOR	receedana	TCTTCTAGAT	
<u>;</u>	TTTCCACTGA			CGTATTCGGG	CARACATOR ACCTECATOR		TOSTICACIONO TOGANGTACO	TCGAAGTACG	ACGCCCCACC	ACAACATICTIA	
101	GATTCAGTCG		CTOSCOTO	GTGCCTANA	ATCHUTTING GTAGCAAGCT		GATTGCCAGG	GGCAGGCCCT	TOOTSTANGE	CTTTACAAN:	
	CTAGGTCAGC				TACATAAAGT	CATEGITICGA	CTAACGGTCC	CCGTCCGGGA	ACCACATTICA	CAMICITIK	
1061	COSTTANGET	_	CATACGETORS	GATATICAGAT	GCATCTTTSA	CIGINITAL	ACCITIOGCTA	TOTTCCCAGC	CATATCCCTC	COCCENTIVA	
1	OCCAATITEGA	_	GTATCCACCC	CTATACTYTA	COTATARCCT	GACATAAAA	TCCAACCGAT ACAAGGGTCG	ACAAGGGTCG	GTATAGGGAG	GCCCCTANGT	
1001	TOTTOTOCAG	-	ACAGTGTATC	CCCTCACTT	GREAMATTHG	TCATGTAGCT		TOCCTOGAAG		COCCULIGIN	
	ACAACACGIC				CCCTTTANAC	AGTACATOGA	ATCTTCCTTT	ACCCACCTTC	TIGAACCICT	GCGGGAACAC	
1101	ACCTICAAGA	-	APPEGRECAT	AATTAATGGCA	ATTARACTICAC	ವಿರುವಿಬಲವಿಬದು	CTYSCCGANG			GTCATAGTTG	
	TOGAGGTTCT	_	-		TACCOGGIG	בכבטבכנכפ	GACCCGCTTC	TATAAAGACC	CTAGICATIO	CAGTATCAAC	
1201	TOTTCCAGGA	•	. ATAXXXXATT	TTTACAAAGC	GCCACHCCACAG	CHITCHCAN				GCGTAGTTA	
)	ACAAGGICCT	-	_	MATGITTE	COCCCGCCTC	CCACOGICTG	ACCCCATATE	ACCAAGGTAG	OCCOGENCIC	CCCATCAA'n:	
1101	CONTRACACIAN	TRICATTICC	CACCETTIGA	GTTCAGATOG	CHANTONICATE	TCTACCTGCG	GGGCGATTAA	GAAAACOSTT	rccocotac	OCCIVITATION	
	OGAGTGTCTA	-			CCCCTAGTAC	AGATGGACGC	CCCCCTACTT	CTTTTCCCAA	AGGCCCCATC	CCCTCTAGTC	
										Psil	
1401	CTYSTERAGRA	ACCAGOTTCC		TONGCAGETE CONCETARICG CARECTOSTICS GEOEGENAAT CACACETATT ACCOCETICA	CAGETGGTTG	GCCCGTNANT	CACACCTATT	ACCORCTIGGA		AACACACACTT:	
	GACCCFICT			GCTCAATGGC	פכדיה אחינים היה המכיכ הכי	CGGCCATTTA	GTGTGGATAA TGGCCGACGT	TOOCCOACOT	TGACCATCAA	TTCTCTCGAC	
	Pst		- •								
1501	CAOCTOCCGT	CATCCCTGAG	3 CAGGGGGGGCC	. ACTICGITAN	GCATGTCCCT	CACTCCTCATAG		CCAAATCCGC	CAGNAGGCGC		
	GTCCACCOCA	GTAGGGACTC	ב פעכנינינינינים	TCAACCAATT	CCTACACACA	CHEMOCOTAC		GGTTTAGGCG	GICTICCOCG	ACCTCCCCCCT"	
							Spltd				
4601	OCCUPANCAG	: TTCTTGCAAG							CCAAGCAGTT	CCAGGCGGTC	
	CCCTATCOTC	: ANGANCOTTC	C CTTCGTTTCA	N ANANGTTICCC	MAACTCTTAC					ייייייייייייייייייייייייייייייייייייייי	
4701	CCACAGCTCG	<b>STORECTOCT</b>									
	GOTOTOGAGO	: CAGTGGACGA	A GATCCCCTAG	ACCTACKTICE	TATAGAGAGAG						
4801	CCAGACGGG			ירכדודוככאכה המאימראהממים				COCCIOCCCT	CCCAGGCTCCC	CCC TG(ACC).	
ı	GGICTGCCCG	3 GICCCAGTAC	C AGANAGGTGC	: הכמכפורכנית	CCACCAGTCG	CATCAGACCC	אייונייר ראריי				

figure 15c

# pMRKAdSgag MER682.

4901		AGGCTOGTCC	TOCTOOTECT O	CAACACATING (	COCTETTEGE E	הנידתה הבחים הדיאנים בחיבות	CCCACATAG C	CATTIGACCA 1	TECTOTOATA O	GTCCAGGGGG
								TCACCCCTA (	GAGCTINGGC	GCGAGAAATA
2001	TCCGCGGGT	COSCCIPINAC	CCCCAGCTIG C						CTCGAACCCG	CGCTCTTTAT
1						CATHERACIA (	CCCAGGTTAAG (	CTCTCCCCCT	TCCAGGGTCAA	AMCCAGGIT
5101	GCCTAAGGCC	CCTCATCCGT						GAGACCGGCA	AGCCCCAGTT TTT	TTTGGTCCA:
					A The Action Court of	GREGETE	GCTGACGAAA 1	AGGCTGTCCG	TOTOCCCOTA TACAMATT	TACACIACTTVI
5201	NCCCCCA16C	AAAACTACO	CAAAGAATGG						ACAGGGGCAT	ATCTCTGAM:
										•
1013	. Academic Contrast	CCTCGAGGGG	TGTTCC(ACC)	TETECTEGE	ATAGAMACTC	GOACCACTCT	_		GCCAGCACG	AAGGAGGCTA
100	TURNING ALL		ACANGGCCCC	ARCAGGAGCA	TATCTFIXEAG	CCTINGTIGAGA	CICIOTITCC	DAGCCCAGCT	cceanceme	TECTCORAT
5401	Actional			GGGGGTCCAC	TUTCHCAGG	MICTOAAGAC	ACATOTOCC (		TCANGGAAGO	TCIATTCASTIT
1080	TCACCCTCCC		AACAGGTGAT	CCCCCAGGTG	NGCGAGGTCC	CACACTTCTG	TOTACAGCOG		AGTICCITICC	ACTANCCANA
5501	CTACCITATE		COGORGITICS	TGAAGGGGGG	CTATAAAAGG	GACTGGGGGC			CCGCATCGCT	GTCTGCGAGG
	CATCCACATC		GCCCACAAGG	ACTITICCCCCC	GATATITICS	CCCACCCCCG	CCCMGCAGG		GGCGTAGCGA	CAGACGCTACC
5601	OCCAGCIOTY	_	CICCCICIGA	ANAGCOGGCA					COATTTOATA	THE ACCEPTORY
	COGTECACAA	- :	GARBERGACT	THYCGCCCGT	ACTGNAGACG	COATTCTANC	<u>.</u> .	TITIGGICCE	CCTAMACTAT	ANGINGACCTI
							Anticipa			
1010	PACTE STATE OF	_	SAMPLES TERRECOCAT	CCATCTOSTC	AGNANAGACA		ATCTITITION TOTCANGUT GGTGGCAAAC			はままたいずれられる
70/6	CCCCCO CACTA	_		GCTAGACCAG			ACAGITCGNA CCACCOITTIG		CROOCCATCT	CCCCCAACCT
					Pwd					
100		CATTA MATERIA ROC	GCAGGGTTTG	GITTINGICO	CGATCAGCGC	GCTCCTTGGC	CGCCATGITI	ACCTOCACGE		AACCCACCCK.
1000	CHICATICAAC	_	COTCCCAAAC	CANAANCAGE	CCTACCCGCG	CCAGGAAACCG	GCGCTACAAA	TOGACOTOCA		Tracerace
1007	CATTIVISMA			GOCACCAGGT	CCACCCCCCA	ACCREGGGTTG	TCCAGGGTGA	CANODICAAC	CCTOOTCOCT	ACCICIOCO
	GTANGCCCTT			CCGTGGTCCA	COTOCOCOCT	TOTOCOCONO	ACCITCCCACT	GINCCAGTIG	COACCACCOA	TOTACAGG
6001	GTACHCOCTC	: GITGGICCAG	CAGAGGCGGC	COCCCTINGCG		GGCGTTAGG	GGTCTAGCTG	CONCINCETEC	GGGGGGTCTG	CONCCACTOR
; ; ;	CATCCGCGAG	S CANCCAGGTC	מיכירכים	GCGGGCMCTC	CCTCCTCCTTA	CCCCCATCCC	CCNGATCGAC	GCAGAGCAGG		OCOCO TOTAL STATE
6101	AAAGACCCCG	3 GOCAGCAGGC	GCGCGTCGAA	GTAGTCTATC		GCAACTCTAG	ממכרומכועפכ			CCCCCCATA
t > •	777777000000		COCCCAGCTT	CATCAGATAG	AACGTAGGAA	CCTTCAGATC	GCCACACGACG			COCOMOCALIA
6201	Ī	_	1 TOGCATECTO	-			ATGTCGTANA	CCTAGAGGGG	CTCTCTGAGT	TARGGTTCTA
		: ccccrogoot	P ACCGTACCCC	ACCENCINGE			ו ו אראפרעו.	פרשורורירי		- Landing Co.
6301		Ξ.	CCGCGGATGC	-			AGGGAGCGAG		CCGAGOTTGC	TAIT (AGINE GEV.)
		r cotagagor	P GGCGCCTACG	ACCOCCCCTG	CATTAGCATA	TCAAGCACGC				OPCACCTANCE.
6401	Ī	_	A TCTGCCTGAA		GACTTYCATG					CHANCE INC.
· •		_	-		CTACCCTACA CTCAACCTAC	TATACCAACC	TOCOACCITIC	TGCAACTTCG	ACCGCAGACA	CICIMMINE

Figure 150

6501	GCOTCACGCA	CGAAGGAGGC	GTACKIAGTCG	CCACACCTTCAT	TGACCAGCTC ACTCGTCGAGG	מנונית אל אל מים המים המים המים המים המים המים המים	THEACHERTETA I	CCCCCCACTA C	GTCCAGGGTT	TCCTTGATGA
6601	TOTCATACIT	ATCCTGTCCC	TITITITIEC		CAACTCCTGT		CCAGANAGGT	CATGAGAACC	ATCCCAAACC TAGCCTTTGG	CCTCOCCT+ CCTCCT+ CCTCCT+ CCTCCT+ CCTCCT+ CCTCT+ CC
6701	CGAACGGTAA	GAGCCTAGCA	TGTAGAACTG ACATCTTGAC	GTTGACGGCC	Truthanche Accept	ACCUTACOCITY Textitacida	TTCTACCGGT ANGATGCCCA	AGCGCGTATG TCGCGCATAC	CCTGCGCGGC	CTTCCGGAC
6801	GAGOTOTOGO	TGAGCGCAAA	GOTOTOCCTG	ACCATGACTT	TGAGGETACTYS ACTECATYSAC	CATAMETIC	TCAGTGTCGT	CCCATCCGCC	CTGCTCCCAG	AGCAAAAAGT TCGTT/TTCA
6901	CCOTCCCCTT	AAACCTTGCG	GGATTTGGCA	CCCCCTTCCA	CTGTAGCAAC	AAGAGTATCT	THY CYCCICCO	ACCCATAAAG	TTGCGTGTGA	TOCKGANGGR
7001	TCCCGGCACC AGGGCCGTGG	TCGGAACGGT ACCCTTGCCA	TOTTANTTAC	CHAGACGACA	AGGACGATET	CCTCAAACCC	GTTGATCTTG	TCCCCACAA	TETAAAGITIC ACATITICAAG	CAAGAAGCGT GFTCTTVGCT
7101	CCCTACGGGA	TENTOGNAGO ACTACCTTCC	CAATTTTTTA	AGTICCTCGT TCAAGGAGGA	AGGTGAGTTC TCCACTCGAG	TTCARGOGAG AAGTCCCCTC	CTCACCCCCT	CCTCTCAAAG CGAGACTTTC	CCGGGTCAGA	GCAAGATKAG COTTCTACTK:
7201	CCAACCTICG	-	CICCACAGGT	CACAGGGCAT	TAGCATTECC	ARGINGGICAC TECACEAGEG	GAMAGETECT	AMCTOGOGA	CCTATGGCCA	TTTTTTCTGG AAAAAGACC
7301	CCACTACOTC	TACMOGRAA: ATCTTCCATE	OCCORPCTO CGCCCAGAAC	TTCCCAGCGG AAGGGTCGCC	TCCCATCCAA AGGGTAGGTT	CCANOGCCG	TAGGATCACGC	GCGCCAGTCA	CTAGAGGCTC	ATCTCCCCCG TAGAGGCGGC
7401	AACTICATGA			TRCTTCCCAA ACGAAOGGTT	AGGCCCCCCAT TCCGCGGGTA	CCAAGTATAG GGTTCATATC	GTCTCTACAT	CGTAGGTGAC	AAAGAGACGC TTTCTCTGCG	TCGGTGCGAA AGCCACGCT
7501	CATGCGAGCC	•	AACTGGATCT	CCCGCCACCA	ATTOCAGGAG	TYSCTATICA	TCTCCTCTTT	OTAGAAGHCC CATCTTCAGG	CHOCCEACOGO	CCGAACACTC
7601	CACGACCGAA				TGCACTXXGT ACGTXXCCC0A				CGCGCACAAG	GAAGCAGAGT
17701	COCAATTTOA	A COCCUTOCC	TOGCAGITY	CACTGGTCAGT	CTTCTACTTC	CACCINGENCE	CCTTOACCGT	CIRACTOCIC GACCOGACT	GACCOCACTT	ACCOPYCAIV:
7801	CCTOCTOCTO							GCGCAGATGG	GAGCTGTCCA	TOGICTCOAC
1901	CTCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	XC OTCAGOTCAG XG CAGTCCAGTC	GEGGGAGETE	CTGCACGTTT GACGTCCAAA	ACCTCOCATA		GACCAGICAG GACGCGFGCT CTCCCCAGIC CCCCCGA	AGATCCAGGT TCTAGGTCCA	GATACCTAAT CTATGGATTA	Trechoode
8001	TOSTITOSTOS	NOSTINGTUS CUGCUTCGAT ACCAACCACC GCCGCAGCTA	GOCTTOCANG		AGGCGCATTC CCCGCCACGCGCGCGCGCGCGCGCGCGCGCGCGCGCGC		מובטבטבטבטבט מו גילעובעונט	GCCGCACCCG	COCCOCCCCAC	TCCTTCCATC: ACGACCTAC

# PMRKAJSGAQ MERGB2

8101	ATCCATCTAA	AAGCGGTGAC	GCGCCCGMGC	CCCCGGACCT	ACTES Y KATOT TOTAL Y KATOTA	CCTSACCCAT CCGGAGARAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGA			COTCORCOCC (CCACCOCC)	GCGCGCGGT#; CGCGCGCCCT!
8201	ASSAGETAGE	OCTOCOCCC	PAGGETTOCTG		נינואנדארוזיהיה	GTTCATCTCC .			_	מנוכנונאשוניא
	TCCTCGACCA	COACGCGCGC	ATCCAACCAC	CGCTTCCCT	מכונטטטטטנ	CAACTAGAGG				כנממככענו
8301	CGAACTTOGA	CTTTCTCTCA	TCGACACAAT AGCTGTCTTA	CANTITICAGE GETTAAAGCCA	CACKTITICACIS CACKTANCING	במבנימפאבנים ו	CCTTTTAGAG	CTGCACGTCT (	CCTCACTTOT	CTTCNTAGG" GAACTATCC":
				HgH						
8401	GATCTCGGCC	ATGAACTOCT	CUATCITCITIC		CHUCHRICAGA TCTCCGGGTC	נישיניוונישכווכ				GCCCATGAC
	CTAGAGCCGG		GCTAGAGAAG		GARGAUCTET AGARGEGRAG	GCCCAGCGAG	CINSCEACEGE	CGCTCCAGCA		CCCNTACTC:
8501	•			CAGACGCGGC		GUCCECTITOS	GCATCGCGG	COCCICATION	CACCTGCGCG	ACATTICAGE F
	_			פוריוטנפנכני						TAACCCAGC
8601	CCACGTGCCG	CCCCTTCTCC	CCCATCAAAG	CONCECCOORC	TTTCTCCATC	MCTCCCACC				ATTGGGTCK:
	•	•								
8701	TCCCAACGTG		GATTCOTTGA TATCCCCCAA	<b>BBCCTCAAGG</b>		CCTTCTAGAA				CCCCCCGAC
)	_		ATAGGGGGTT	CCGCAGTTCC	CCGAGGTACC	GGAGCATCTT	CAGGTGCCGC	TICAACTITI	TGACCCTCAA	CCCCCGGCTG
8801		· CCTCCTCCAG	AAGACGGATG	AGCTCGGCGA	CAGTOTCOCG	CACCTCCCGC				TCAATCTCE
: ) )				TCGAGCCGCT	GTCACAGCGC	GTCGAGCGCG	AGTITICCGAT	GTCCCCCGGAG	AACIAACAAGA	ACTTAGAGGA
					_				E STATE OF THE STA	1
1000	CHATCATAAG	TO THE PERSON AND ADDRESS OF THE PERSON AND	TCTTCTTCTT	CTOCCOCCG	TEGREGAGEG	GCRGACACCGC	GGCGACGACG			CAMACGCTC
					ACCERCATER	CCCTATACCG	CCGCTGCTGC	COCOTOOCCC		GTTTCGCGAG
1000	Ī	CCCCCCCCAC		CTCGGTGACG	Granascar	111000000	GCACAGETOS		CCGTCATGIC	CCCASTTATES
) ) )					כפנפנכנוטכע	AGAGCGCCC	CCCCTCAACC	ידיכידטכסמכם	GCCAGTACAG	GCCCANTACC:
9101	Ī	3 GOCTGCCATG	CGGCAGGGAT	ACCCCCTA	CGATCGATCT	CAACAATTGT	TUTTETAGGTA		GACCCACCTO	AGCGAGTCC
l l	CAACCGCC		GCCGTCCCTA	TOCCOCONT	CCTACCTAGA	GITGITAACA	ACACATCCAT	DACCCCCCCCC	CTCCCTGGAC	TCGCTCAGG
			Xhol							
9201	CATCUACCOS	3 ATCGGAAAC	·E	AGGCGTCTAA					OGCNGCGGGC	acconcas:
		TAGCCTITIO	GAGAGCTCTT	TCCGCAGATT	GGTCAGTGTC	ACCUTECAT	CCCACTCGTG	GCACCGCCCG	ccercecces	CCGCCAGCCT:
9101	GITGITICIO	3 OCCIONACTOC	TOCTGATGAT	GTANTTANG	TACKACYACTUCT			AGAAGCACCA	TATICCTTGGG	ירכנאפנריופנ
			ACGACTACTA	CATTAATTTC	ATCCCACAGA	ACTUTOCOGO	CTACCAGCTG	TCTTCGTGGT	ACAGGAACCC	AGGCCGGACG
9401	TOAATGCGCA	A OOCOGITCGGC	: CATTACCCCAG	GCTTCGTTTT				GCATGAGCCT	TICTACCOGC	ACTTOTACT
	ACTITACGCGT	P CCCCCAGCCG	GTACCOCCTC	CGANGCANAA	CTOTARCOCC	CHCCAGAAAC			AAGATGGCCO	TENNINAGA
9501	1 CICCINCCIC	TTUTCTOCA	A TUTTETTICAT						CCCATGCOTO	TOACCCCOAN
	GAGGANGGAG	3 AACAGGACGT	T AGAGAACGTA	ו האדאמיכה מכם	נתיהכניוכניפכ					NC I GOOD C
9601	I GCCCCTCATC	C GCCTGAAGCA	N CHARCTAGGTC							ATCCATCTCT.
٠,	COGOGAGTAG	3 ccaacificat	r cccgatccag	CCCCTGTTGC	GCGAGCCGAT	TATACCAGAC	GACGTGGACG	CACTCCCATC	TGACCTICAG	TALASIALAD

Figure 15F

9701 ACAMACCOT GGTATOCCC CONTITIONING GREENANCE ACTIVITIES ANCIENTAL TRACEGICS GGGTAGGGC CTGCGAGAGC TCGTFGARC	TOTITIOGCA CCATACOCGO CCACAACTAC (ANTATHEMEN TEACCOMITA THEFTOGOTA ANTIGOCOACA CCACTOROCC GACACTOTICG AGCCACATOM	
CTGCCIACAGC	CACCICITCI	
GOTTABCCCGG	CCACTOROCC	
TTAACGGTCT	AATTGCCAGA	
AACGCAAG	THEFT	
AGETTEGGCCAT	TCAACCONITA	
GTTETAACTTE	CATATITIACE	
CGTGTTGATG	GCACAACTAC	•
GGTATGCGCC	CCATACCCGG	5
ACABAGCGCT	TOTTTCGCCA	
9701	5	

2,000	GENCHAGACE CHOCAAAAGA ACACCCTICHA AGCASTACH CTHECGINGH CHATINAGATA AATHUUCAAG GOIMICATUS COONCONCH	CONDITION CACCITITICS TOTOGROUP TOSCUCTION GARGACTAN THANGOSTIC CONTROLAGE CONTINUED CONTINUED CONTINUED CONTINUED CONTINUED TO CONTINUE CONTINUED	COCGIANICO GOOGICOTO GIANICCATO CONTINCOSO COCONTINTO ANCOLAGIO NOCOROGICA GACARODOS GAGIOCITOS	GOOGATAGGE CECENGECTS CACTAGGTAC GCCANTGATG GACTICACAG THARTECAC ACCTECAGA CTGTTGCCC CTCAGANGA	PHYCHARIOG OCCIONATI CCCOTACTY TITTERICAC TRICCOCCO CHICTANGE GOTTAGGETO GAAGEGAAA GCATTANETH:	ANGRECISCO COCCONCA COCGATICAA AAAACOSTO AACOGGGG GTOGCATICO CCAATCOAC CTITOGCTIT COTAATICAC	PERSONNEL GERTFATTIFF CCARGOTHS AGHICCOCKING CCCCGGTTC CACTCTCGA CCGGCCGAAC GGGGGTTTGF	ACATICOCCT CCCANTAAA GOTTCCCAAC TENACGCCCT GORGGCANG CTCAGAGCCT GOCGGGCTTG CCCCCANDOL	Control of the state of the sta
٠	GCGGGAYACT CTACCGAGGT CTG	YEACCCTITIA GRACICIACTA GAC	CONTINUES CONCENTING AND	פכתאדנאמים מהכנוכאכאפכ דדמ	HTTREECAC TRECCONCRG CAG	WAAACCOSTIG ACCOGGGGG GTC	AGINCACARA CCCCCGGTTC CAG	ובאזכטככנד מסאממככאאם כדכ	
annual and a second a second and a second an	CHCTAGACC OTOCAAAAGG AGAGCCTGTA A	GAGATCTOG CACOTTTTCC TCTCGGACAT T	CCGTATCCG GCCGTCCTCC GTCATCCATG C	GOCATAGGC CGCCAGGCCC CACTAGGTAC G	THE MERCINE CONTROL OF CONTROL T	AGGIECTEC COCCENCIA COCCAICEAN N	VIRGORICAS CASTITUDATITUD CCARGOSTICS A	CATCOCCT CCCANTAAAA GGTTCCCAAC 1	
	10101 AATCOTTGAC OC	TTAGCAACTG	10201 GGOTTCGAGC CC	CCCAAGCTCG		BAACTGAAGG		CONCORPOR	

10501	CTCCCCGTCA	TOCANGACCC	COCTICCAR	Trechecoga	ACACCACC	AGCCCCTTTT	TRUTTUCE	CABATCARIC	251251351	ocupanion of
•	GAGGGGCAGT	ACGITICAGG	CCGACCTTT	AAGGAGGCCT	THETCCTIGG	TCGGGGAAAA	AACGAAAAGG	CTCTACGTAG	GCCACGACGC	CONCINCOC
10601		ACHCACACACA	AGACCAAGAG	CAGCGGCAGA	CATCCAGGGC	ACCTCCCTT	CCTCCTACCG	COTCAGGAGG	GCCGACATCC	<b>GCGGTTGACK</b>
1	GOOOGAGGAG	recreacear	TCTCTTCTC	GTCGCCGTCT	GOOGRAGAS TESTEGECOT TESTEGISTICS GIEGECOSES GIAGORICARIA GIAGORICARE GEAGIECTEC CEGESTARGO COCENACIVAL	TYNGGARGERGA	GGAGGATGGC	GCAGTCCTCC	CCCCTGTAGG	CCCCMCTVK
10701	COCCAGCAGA	TOGTOATTAC	GAACCCCCCGC	COCUCCOCC	CCCCCACTAC	CTRGACTTOG	ACCAGGGCGA	9000010000	COCCTACION	COCCCINCTO.
	accencence	ACCACTAATG	CTTGGGGGGG	CCACAGCCCA	GCCCTCATG	CACCTCAACC	Treteceet	CCCTAGNCCCC	GCCGATCCTC	GCGGGACA(*;
10801	TIME	CCAAGGGTGC	AGCTGAAGCG	TOATACGCGT	CACCCCTACC	TOCCOCCOCA	GAACCTRATT	CGCGACCGCG	ADGGAGAGGA	GUCCESACIONS
	ACTOGCOOTO	OCTTOCCCACG	TCGACTTCGC	ACTATGCGCA	CICCOCATRIC	ACGGCCCCCT	CITICKGACANA	acachagooc	recenence	COGOCHCCTC
10001	ATTENDED	GAAAGTTCCA	CGCANGGCGC	GAGCTOCCARC	ATRICICATION	TREACGARCERS	THICTIGGIGG	AGGAGGACTT	TOAGCCCGAC	GCGCGAACCG
10601	TACCCCTAG	CTTTCAAGGT	GCOTCCCGCG	CTCGACGCCG	TACCECETAG CHITCHAGGI GCOTCCCGCG CITCAACGCCG TACCAGACTI AGGGCTCGCC AACGACGCGC TCCTCTGAA ACTCGGGGCTG CGCGCTTGGC	AGCGCTCGCC	AACGACCCCC	TCCTCCTGAA	ACTOGGGCTO	COCOUNTGE

TITICAAAAA GCTTTAACA	AAAGITITIT CGAAATICIT	AMANCCCAAA TAGCAAKKTO	SEA PASSABLACE GEOGRAPHER CONCERNATION TARACTURES CARRECTED TITTEGRATT ATCHTEGRA	THE ACTIVITION TATABLES CACAGEAGA ACAAPGACH, ATTEACHAT GERTECTAA ACATAGTAGA GEOUGAGGE COCTOGETEC	THE REPORT WITH THE PROPERTY CHIPMOTICS TRACECTED TRACECTED COCCAST TOTAL CASCIFICATE COCCUCCO GCGACGACT
HANCH GGAGATTANC	TTOGT CCICTANTIO	TANGE GEGETGGAGE	NATITE CECEACETES	NICTAN ACATAGTAGA	ACCAPT TGTATCATCT
TACCIANTEANA COUTO	ATCCTACCTE GLEAC	ATCTUTE AND CTITE	TAGACACCCT GAAAC	אזידכאמאיד הניתיד	TANGTCCCTA CGCGA
COACCT COTAACCACA	ACTION CCATTIGGORT	CCTATA GGACTICATIC	CGATAT CCTGACTA:G	IGCARRE ACAACGACAR	MERCEL TRANSCACE
	CHACACCOCC COCC	TRANSPORTED TO THE	CCCCCTCCT CCAC	TATAGTGCAG CACA	Line . Li
CONTRACTOR CARCINISTICS CONTRACTOR CONTRACTOR CONTRACTOR CONTRACTOR CONTRACT THEADANA CONTRACT	CETABLE CONTRACTOR CONTROL CONTROL CANTROCK AND ANGINEDIC CONTROL CONT	CONTRACTOR BATTERIANS CONTRACTOR CONTRACTOR CONTRACTOR ANCHORISM CONTRACTOR CONTRACTOR ANACCONA INCOMENCE	GETTER TREESPACE	CTF ATRICINET AGENCITION	The state of the s
1001		11101		11201	10911

		Z-A	T T T T T T T T T T T T T T T T T T T							
11301	TEGATIFICAT	MACATECTG CAGAGGATA		TGCTCAGGA	נונינונ. איני דדנו	אינידידאיזהרדי	אכאאנאטנאניר		TATTCCATGC	TTABCCTGGA
	AGCTAAACTA	TITICITAGGAC	GICTICITATIC	ACCAC: FICCT	CONTRACTOR	TUTEAUTORAC	TESTACEO		ATAAGGTACG	AATCGGACCC
11401	CAAGITTITAC	: GCCCGCAAGA	TATACCATAC	CCCTTACGFT	CUCATACACA	ACKING TANA	いろれいいしょうしょう	TTCTACATGC	CCATCCCCCT	CAAGGTTGT '
	GITCAAAATG		ATATCCTATC		GCT:TATKTIFT	TTTANTACAT	CTARACTECNIC	AAGAINTACO	COTACCGCGA	CTTCCACG/ 1
11501	ACCTTGAGGG		CGTTTATCGC	MCGACACACA	TIXEACTANGGE	בנידומאיינפדוני	Arccondac	GCGAGCTCAG	CONCCOCUNG	CITSATTSCACA
•	TGGAACTCGC		GCAANTANCO		ACKSTICTTICGG	GCACTCGCAC	TCAGCCGCCG	COCTCGAGTC	GCTGGCCCCTC	GACTACOTATE
11601	CCCTGCAAG	1 GOCCCTGGCT	GGCATGGGCA	GCGCCCATAG	NUNCCUCANG	TCCTACTTTG	ACGCGGGCGC	TRACCTRACGC	TOGGCCCCCAA	GCCCAACTCGC
	COGACOTIME		CCGINCCCGT		TETECOGETE	AGGATGAAAC	TOCGCCCGCG	ACTEGACOCO	ACCEGGGGGTT	COSCLECCO:
11701	CCTCGCAGGC	A GCTGGGGCCG	GACCTGGGCT	GGCGGTGGCA	בבבבינובבינ	CTCGCAACCF	COCCGCCTG		ACGAGGACGA	TGACTACGAG
	GGACCTCCGF		CTGGACCCGA	CCCCCACCGT	20202020000	GACCGTTGCA	DUCCCCCCCAC	CICCITIAIN	recreereer	ACTICATICCTIC
					-				Psil	
11801	CCAGAGGACG	3 GCGAGTACTA	Accortonto	THETGATEA			ACCCGGCGGG		CTGCAGAGCC	ACCCOTCC()
l I	<b>GENETICE TIGG</b>	C COCTCATGAT	TCGCCACTAC	AMGACTAGT	CTACTACGTT	CTGGGGTTTGCC	TOGGCCCASCA	כפכבכנסכנפכ	GACGICTCGG	TCGGCAGGC
11901	CCTTAACTCC	C ACGGACGACT	OCCCCACG	CATGGACCGC	ATCATGTCGC	THACTRECARG	CAATCCTVIAC		AGCAGCCGCA	GGCCNACCG
1	COAATTOACO		_	GTACCTGGCG	TAGTACAGCG	ACTGACGCGC	GTTAGGM:TO	CCCANGOCCG	remedeedt	CCOCHIQCC
							Paris	•		
10001		A THY THE SABORC	GENOTECEG	GCGCGCGCAA	ACCCCACGCA	CGAGANGOTG	CTOGCGATCG	TAMACOCCCT	GGCCGAAAAC	ACCIOCCATICIT
10021	CA			CGCGCGCGTT			GACCGCTAGC	ATTITICGCGA	CCGGCTTTTG	TCCCCGUTAG
10101					CCCCTGGCT	CGTTACAACA	GUGGLAANGT	GCAGACCAAC	CTYGACCGGC	TOTOTOGGGG
10171	GOLLLONICO				ACTION ACTION	CLAATHUTTUL	CGCCGTTGCA	CCTCTGGTTG	GACCTGGCCG	ACCACCCCT"
	CCOOCCIOCT	T CCGGCCGGAC			בסרותרונות					
12201	TOTOCOCCAA	a occaracea	-		CAGGGCAACC	TOCCOLOCIAL	GGTTGCACTA	AACOCCIFICO	TOMOTACACA	SCCCCCAM.
	ACACGCCCTC	C COCCACCOCO	TCCCACTCGC	GCCCOTCGTC	GICCCGTTGG	ACCCCAAGGTA	CCAACGTGAT	TTGCGG/MGG	ACICATGIOE	COCCIOCOTAL
12101	OFFICEORGO	G GACAGGAGGA	CTACACCAAC	THURKINGCO	CACTGCGGCT	AATGGTGACT	מאלאכאכנפכ	AAACTCAGGT	GTACCAGTCT	GOCCCAGACT
	CACTAGORCE			GATGTGGTTG AACACTCGC	CTRACCCCRA	TTACCACTGA	CTCTGTGGGG	TITICACTCCA	CATGGTCAGA	CCCCCTCTCA
				_						
					Critichia	CITTITIONA	ACTIGGARG	OCTOTOCOGOG	GIGCGGGCIC	CCACAGGGGA
10621	AFTITION			TACABOTO PARTIES			TGAACGTCCC	CCIACACCCCC	CACGCCCGAG	CONCINCOCCT
	Southway I				CHARTECTIC	TECTANTAGE	CALICATION	GACAGTGGCA	GCOTGTCCCG	GGACACATAC
10521	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC				GACANCGACG	ACGATTATCG	CGCCACAGTCC	CTGTCACCGT	CGCACAGGGC	CCTGTGTATG
•						CCACCAGCAT	ACTITICGAGG	AGATTACAAG	TOTCAGCCOC	מכמכשמפנים
10071							THIMAGGICC	TCTAATGTTC	ACAGTCGGCG	COCCUVCCCC
	פאורנאסופ							•	Pirrel	
1921		ر ممردهمدين	CAGGCAACTC	TAAACTACCT	CHILANTIAAC	CARCAGEAGA	AGATECECTE		GPTGCACAGT TTAAACAGCG	
***	The The That Is		_			OCCURRENCE.	TUTACKAGAG	CAACGTGTCA	ANTINOTICUC	TCCTCCTXXXX
					ATI STRUCTS	נות אבו אינטינים	CARCETGGG	CTRGACATGA	הכמכככה	CATGGAACCG
12801	CATTITIOCOC								GGCGCGTT	GTACCTTGGC
•	A STANDARD									
			•					•		٠

Figure 15H

, 200	Control of the control			AACACCCCPAAA	Try: Ac. P. Acres	מראדת היים	Christian	ACCCCCAGTA '	TTTCACCAAT (	CCCATCTICA
10671	GICATGERIO			ANCE THE STATE	A COURT BOOK				AAAGTGGTTA	CXXTAGAACT
	CCCTACATAC	GGAGTTTGGC	CCCCAAATAG	TRAJERATE METRIAN	M. H.A.H.A.					
13001	ACCCGCACTO	GCTACCGCCC (	<b>ECTORITY IN THE PARTY OF THE P</b>	NCACC(SGR)	ACACCURENT: ATTCCACHITC FOUGACTATTA	רנייה התראשהדת				וויטוילין ווישלאל
1	TETTETT		GGACCAAAGA	TYTHINGCCCCC	TAAGCTICCAC	TAAGCTICGAG GCACTICGAT TGCTACCTAA		CHARGACCCTG	CTOTATCTGC	TOTOCOACAA
								HingRid		
					THE ACTION ACTIONS	Acceptance .	GCGAAAGGAA AGCTTCCOCA		GGCCAAGCAG	CTICTCCGAT
13101	TICCCOCA	ברותרשונים	SC I WANTED	CATTER CATCOLO		W.Y.D.J.EJ.Y.H	CHETTECTT TEGMOGEGT			GAACAGGCTV.
	AMERICALIA								•	
.025			CHERCAGATECT	ACTACATOR	TTCCAALETT GATAGESTCT		CTTACCAGCA	CTCCCACCAC	CCCCCCCCCCC	CTOCTORGO:
13501	GATTORICAL			TCATCGGGTA	AAGGTTCC:NA		GAATGGTCGT	GAATEGICGT GAGCOTGOTO GGCGGGCCC		CACCACCC
			Psff	_						
13301	ADGAGGAGTA	CCTAAACAAC	TEGETISCING	<b>דכטכדוטכו</b>	CGANAMARC	כשמכבשבנימפ	CATTICCCA		GAGAGCCTAG	TOGACAN!AT
1	TCCTCCTCAT	COATTROTTO		TOCOCOTOCO	GCTTTTTTG	GACTIGARTICC	GTAMAGGGTT	GITGCCCTAT	CICICOGAIC	ACC'TI :TTCTA
11401	CAGTACATVE	AAGACGTACG	CCCAGGAGGA	CAGGGACGTG	CCAMPETERS	GUCCHACCAC	COGICOTOAA	AGCCACGACC	-	reregreter
10101	CTCATCTACC		GCGTCCTCGT		שנונונולאאכנו	CONTRACTOR	GOCAGCAGTT	recerected	CAGICOCCC	AGACCACACT
11501	CACCACCATO	ACTORDEAGA	CGACAGEAGE	GPCCTGGATT	TICGGACACATAC	TRECARCECE	TTTCCCCACC	TTCGCCCCAG	GCTGGGGAGA	ANCTITIONA
10001	CHYCHIGETAC		GCTGTCGTCG		ACCCTCCC.TC	ACCCITICGGC	NANCOCCITY	<b>AAGCGGGGTC</b>	CGACCCCTCT	TACANATIT
13601					CCATRACACC	GAGCGTTGGT	TITICITISTAT	TCCCCTTAGT	ATGCGGCGCG	COCCGATGTA
TORCY		CONTRACTA			GGTACCCTAGG	CITTECAACCA	ANAGANCATA	AGGGGAATCA	TACGCCCCCC	<b>GCCGCTACAT</b>
,							CATTOROTTE	CCCTTCGATG	CTCCCCTGGA	CCCCCCTTT
13701	TGAGGAAGGT					Arrestricting	CCACCCAAGA		GAGGGGACCT	GGCCCCAA.
	ACTCCTTCCA	5	GGAITACIU	אכארוארוויפ	רפרנטרפוני	Metherope				
		Kori								
13801	<b>OPOCUTODO</b>	GGTACCTIGCO	<b>GCCTACCOM</b>	-						CIGNICACA
	CACCCAGGCG	CCATGGACGC	COGATOCCCC	CCCTCTTIOT	CCTAGGCANT	GACIACTICIAAC				GACCACCTOT
13901	ACANGTCANC	GGATGTGGCA	TECCTIONACT	ACCAGAACGA	CCACACCAAC	THICHENCEA	CGGTCATTCA			GGGAGGCAAG
	TOTTCACITIC	-		TRESTUDEN	GETETEGETTG	AAAGACTGGT	CCCAGTAAGT	TINGTIMENO	ATCITCOGGCC	CCCLCCCLL
14001	CACACAGACC	ATCANTCTTO	ACGACCGGTC	GCACTORGE	CKICCACCTEA	NANCCATCET	GCATACCAAC			CARCITITACC
	GTOTOTOTO		TCCTCCCCAG	CONTRACTOR	CCCICTCGACT	TTTC:CTACK!A	CGTATGGTTG	TACOGITITAC	ACTITICATICAA	GTACAAATTAG
14101		AGGCGCGGGT	GATOCTOTOG	COCTTGCCTA	CTAAGGALAA	TUACITITICAG				CCCCANANACA
	TTATTCAA			: GCGAACGCAT	GATTICCTVITT	AGTECCACYTIC	GACTITATEC	TCACCCACCT	CAAGTGCGAC	GCCCCCC
·				•	Pwil			•		
14201	ACTIFICATION	GACCATGACC	ATAGACCTTA	TOMOMOG	CATCGTGGAG	CACTACTTCA	AAGTGGGCAG			
				P ACTITION Y	CTAGCACCTC	CITCATGAACT	TTCACCCGTC	TOTAL	CAAGACCITT	CACAGAMOCC
14101	Ī			ר המתרדדעית	CCCOTCACTR	CHKTPTKTACAT				TCCAGACATU
	CCATTICA				GOGLAGTIAC	CACAACAGTA	CGGACCCCAT			MACTICITATION
14401		CENTRATECES	1 GGTGGACTTC	: ACCCACACACC	GCCTYCAGCAA	CHICHECANGE				
			: CCACCTGAAG		COCACTECITY	GANCANCCCG	TACKCCTTCG	CCGTTGGGAA	GGTCCTCCCG	ANATCCTAG"

GCCCACCGC CCCCACTCGC ACCCATTCGC ACCCTAAGCG GAGAAGCCTT CTCTTCGGAA	GCAGU	CTACTROTICES GATGACCASC CACTCCAAGA GTGAGGTTCT Asc	AAAACCCCCO	AGTCCAGCGA TCAGGTCGCT			ANATGANGA FFFFACTICI GCACCARCO		RETPARTICE CHICGCHING COGNIGORY COCCCCCC CHARLIANING COLATORICO GACGCCHING GACACOCOTO GACACOCOTO GACACOCOTO GACACOCOTO GACACOCOTO GACACOCOTO CACANACAMA ANGAMACTAT GACACOCOTO TATANGTITE TICTCTACAMACTANA CACATACOTO CACACOCOTO TATANGTITE TICTCTACAMACAMACAMACAMACAMACAMACAMACAMACAM
CTRATCCCGC TRANCGATCA ACTICCTAGT ACCCGAGGTC TRANCCAGG	ACCCAGTACC TGGGTCATGG	ACCTECEGC COCAGCAGE TOGACCCA CCTCCCTCA GCCCCAGCT OTTCCCCGTC CCCGGCTCGA CAACGGCAC	GAGAACCAGA			TTCGCGAGGC CCATCGACGC GGTAGCTGCG	GCGCTATGCT CGCGATACGA CGCGCACGTC	CERCECTOR COCCACAGE GETCHCCORC GGCGTCGTCG	COSTGCCAC CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
AGNTGACACC TCTACTOTGG GTGGAGGACA CACCTCCTGT CCCCTCGCACGCA GGCGACGCCCACGCACGCACGCACGCACGCA	CAGCACCTTC		TCTCTGACCE ACTIOTICAA TCGCTTTCCC GAGAACCAGA TTTTGCCXXX AGAGACTGGG TCCACAAGGT AGCGAAAGGG CTCTTGGTCT AAAACCCC(X)			CGGGGCCAAU GCCCCGGTTC GTCGATGACG CAGCTACTCC	GCGGAGCCCG CGCCTCGGGC CCTGCTTAAC		COSTGCGCAC COSTGCGCAC COSTGCGCAC COSTGCGCAC COSTGCGCAC COSTGCCACCCAC COSTGCGCACCACCACCACCACCACCACCACCACCACCACCACC
	TAAGCAATGA	TCCTGACCTA AGGACTGCAT CCGGGTGGTGG	ACCTICITICAA TOCACAAGTT	CAGATCACGO GACGOTANCO GTCTAGTISCC CTISCOATGGC	THINGARGE CETERRENT GICHEGEEGE ANTITICES RACECETAT CAGAGEGEEG	CCCGGACGG TTCCCAAGCA AGATGTTTGG CCCGGACGG AAGGGTTCTT TCTACAAAGC CACAAACGG GCCGCACTTG GCGCACCTAC GTGTTTGGG CCCGCAGAAC	CCAGNITICA CANTIGACGO GOCCATICAA ACCONOTICO GIFCACCACO CONTINATO TOPICACCACO GOCCACORCA CONCACACACO	GTCCAGGGA GTCCAGGGA CAGGTCCGCT	CTREGERATEC RACGCRIACG ACGAAGCTAT TGCTTCGATA
CCCANTRACTOR CCAGCTTGAA CCGANTRACTOR A CCTCCANCTT CTCCCTTCCTC TTACCTCCTC ACCONTRACT CCACCCCCC	TACAACCTAA	TOCTTITICAC ACGANACGTG CAGCANCTTT GTCGTTGANA			CCTCCCCTAT	CCCGAACGC TTCCCAAGCA CCCGAACGC AAGGGTTCTT CACAAACGC GCCGCACTCT GACATTGCG CCCGTACTCT GACATTGCGC CCCGTGACC	GOCCATTICAG CCGGTAAGTC GCCCAACGCG	TRECECCENG ACTROCOCTEC	
	GATCAAA CCCCTGACAG ANTACAGGAA GAAACGCATT	TCATTGACCC AGTACCTTGG CGCGCTAGAT GCGCGGTCTA	AACTCATCO CCAGTTTACC TTGAGTATAGG			CCCOGACGG CCCOGACGG CACAAACGG	CCAGIVITICA CAGINGAGG GIFCACAIGT GICACCTIGG GCCGCGACC CAGCACTICC	CTGCCGCCTC CGCGCTCGT GCAGCGGTGG GGCGGCTGG GCCGTGACGG CGGTTGACAC SIII ACGGGCGGCC ATACGGGCG CTCGAAGGCT GGCGGCGTGT ATTGTCACTG TTCCCCCCAG TGCCCGCGG TACGCCGGG GAGCTTCCGA CGGCGCCCA TAACAGTTACA CGGCGCCCA	CAGGGTCG CAGARACAAC GTCTATATAS TCCGCGACTC GTCCCCAGC GTCCCCGTTG CACATAANCC ACGCGCGGAAAACTAAAAAAAAAAAAAAAAAAAAAAA
CCCCACTGTT CGATGTGTAC GGCGTGACAA CCTACACTTG CGTTCTCTTG AGGTTGTCTC AAGGTCCTTG AGGTTCACTTCT TTCGCCGAC TCCCACTTCT	APSACAGGAA TCCTGTCGTT	CCCTTACACCA TTCCGCTCCA AAGGCGAGGT	AACTCATCCG TTGAGTAGGC	TGAAAACGTT ACTTTTGCAA	TGCCCCTACG ACGGGGATGC	ACACAGGCTG TGTGTCCGAC CTTGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCG		GCAGCGGTGG CGGCGGCTGG CTCGAAGGCT GGCCGCGGTGT GAGCTTCCGA CCGCGCCCA	AGRACTATOR CTCAGGGTCG CAGGGGAAC GTCTATATCG TCACGATACT GAGTCCCAGC GTCCCCGTTG CACATANCCC TTCCAAGAAA AACTACTTA GACTCGTACT GTTGTATGTA AACGTTCTTT TITGATGAAT CTGAGCATGA CAACATACAT
CCATTACATTC CCATTATATATATATATATATATATATA	CCCCTTACAG ANTACAGGAA GGGGACTGTC TCCTGTCGTT	ACCCTCAGAC TOGGAGTCTG CCCCGTGACC	ACCAGGCC GTCTACTCCC TOGTCCGG CAGATGARGG	CCACCCTICAG	ACGCCCACACC TGCGGCCTGG	CCCAGCAATA GGGTCGTTAT ACCCCACGC	CACOCCACA GRACACCACA CGTCACCACC	GCAGCGGTGG CTCGAAGGCT GAGCTTCCGA	CAGGGGGAAC GTCCCCG1TV GACTCGTACT CTGAGCATGA
TCTGGAGGOT GCTAACATTC AGACCTCCCA CCATTSTAAG AACAGCAGTG GCAHCAGGGG TTGTCGTCAC CGTGGCCGGG TTGCCACACG GCCTGAGGAG AACGGTGTGC CGGACTCCTC	CCACTAGITT	TACGCCG JATGCCGC VTGCAAGA	CONCCAGOCC	CCCACCATCA	CTGACGCCAG	CCTTATATCO GGAATATAGC CGCGGGCACT	ACTACACGC TGATGTGCGG GCGCGTAGCA	CGCGCATCGT SRI ATGCGGGCCG TACGCCCGGC	TCCAGATACT CTCAGGTCG TCCAGATACT GAGTCCCAGC TTCCAGAAA AACTACTTA AACGTTCTTT TTTGATGAAT
CCTACGATUA 1 GGATGCTACT A AGGICGCAGC A TCCGCCGTCG 1 GGCGACACCT 1 CCGCTATGGA A	AGAAACC TCTTTGG	Kiph CCTTGCATAC AACTACGGCG GGAACGTATG TTGATGCCGC TTGCCAGACA TGATGCAAGA AACGGTCTGT ACTACGTTCT	TETACAA	Asei CCCGCCAGCC GGGCGGTCGG	GTOACCATTA	GCATGTCCAT CGTACAGGTA AGTGCGCGTG	GACGGCCGAGGCTAGGGCGAGGGCGAGGCGCGCGCGCGCG	ACOGGGGGGG	AGRECTATGA CT TCACGATACT GA TTCCAAGAAA NA AACCTTCTTT TT
14501 0	14801	14901	15101	15201	15301	15401	15601	15801	15901

Figure 15J

		Bogin	€.							
16101	r-Aragh-Aft	VDWSCOSSOS	THARBITAIT GRECHING TOTALING COCCANGANG CANGARGANG ATTACHARGE LYGAAAGCTA	CCCGAAGAAG	מאמאמנאמני	ATTACAAGCC C		ANGCGGGTCA A	ANANGANAAN	GNANGATGAT
	GOTCCAGTAG	COCOOCCICT	COTCCACTAG COCCACCTICT AGATACCGGG GGCTTCTTC CITYTCGTICC TAATGTTCGG GCCTTTCGAT	OGCITCTIC	CTTY TOSTICE	TAATKITTÜÜ		CCAGT	THICHIT	CTTTCTACT.
16201	GATGATGAAC	TTGACGACGA	CONTRACTOR	CTCCACCCTA	COTHOLARING CITCLACGCTA CITCHICCART CARRACTA			-		I TEACHARTER.
	CTACTACTTO	AACTGCTGCT		GACCITCICGAT	CCACCITICAC GACGINGGAT (XXCCCCCCT), CGCTNCCCTAT					Mac German
16301	GCACCACCGT	AGTETITACO	CCCCGTICAGC	GCTCCACCCG	GETECACCEG CACHTACAAG CIRCHISTATIG					NGCAGGCCAA
  -  - 	COTOGTOGCA	TCAGAMATGC	GGGCCACTCG	CCAACGTVACAC	CHARGER CHORATETTE GENERATAE		TACTCCACAT (	OCCOCTOCTC O	CTGGACGAAC	TCGTCCGGTT
16801	COMPANY	CASCORCITION		GCGCCATANG	מאכיאוייניים המודימנייביד המאכשאפיני	נינונונינינינים י		ANCCCANCAC (	CTAGCCTAAA	GCCCGTAACA
	OCTOCOCOCAC			COCCGTATTC	CTGTACGACC GCAACGGCGA CCTGCTCCCG	GCAACOGCGA		Processor (	GATCGGATTT	COCOCATTICIT
	Pad									Kini
16501	CHACACACACAC	THE TROOPER	GCTTGCACCG		TCCGNAGANA AGGGCGGCT	AAACATACGAG	TYCTEGETYSACT .	TOCCACCCAC COTOCAGCTO	CONOCAG	ATCGTACCCA
1	GACGICCITCC	_			TCGCGCCG3A	PPROGRAM	AGACCACTGA !	ACCGROGOTO	GCACOTCGAC	TACCATATAGET
16601	MOCOCCAGCG		GICTICGAMA	AAATGACCGT	GGAACCTTAXG	CHAGARCCCG	AGGTCCCCGT		AAGCAGGTGG	COCCEOGACT
,	Tracactrac	_		TTTACTGGCA	CCTTTGGACCC	GACCTCGGGC	TCCANGCGCA	COCCOOLITAG	TICGICCACC	GCGCCCTGA
16701	Grandette		TYCAGATACC	CACTACCAGE	AGCACCATTA	THECCACCGC	CACAGAGGGC	ATTOCHONCAC	ANACOTECEC	GGTTGCCTCA
	CCCGCACGTC		_	_	TCGTCGTCAT	AACGGTGGCG	grencicces ,	TACCTCTGTG	THIGCAGGGG	CCAACGGAGT
16801	000000000		_	מכזאניםמככם	CGTCCAAGAC	CTCTACAGAG	GTTSCAAACTG		GTTTCGCGTT	
	COCCACCOCC			_	CCAGGITICIE	GAGATACCTC	CACGITITIZECE	TOCCCACCTA	CAMAGCGCIA	
16901	000000000	CCGTTCGAGG	MAGTACGCC	CCCCCAGCGC	OCTACTOCCC	GANTATGCCC			ACCCCCCGGCT	ATCGTGGCT
	200000000	_		GCCGOTCGCG	CCATTACOO	CTTATACGGG	ATCTAGGAAG	GTAACGCGGA	TGGGGGCCGA	TAGCACCGAT
17001	CACCTACCOC	CCCAGAAGAC	: GAGCAACTAC	CCCACGCCGA	ACCACCACTG	CANCCECTE			CCGTOCTAGC	CCCGAITTCC
	OTOGATOGCO			CTCGTTGATG GGCTGCGGCT	TGGTGGTGAC	CTTGGGGGGG	CCCCCCACCG	GCAGCGGTCG	GGCACGACCG	
17101	GTGCGCAGGG	TOCCTCGCGA		ACCUTOGRAC	ACCOMPANDE ACCOMPANDE TECCANOMIC	CCCTACCAC	CCCAGCATCG		GOTOTHONG	
1	CACOCOTOCC		r recirection	TRACACCACG	Accortored	CACGATGGTG GOCTCGTAGC		ANATHTEGG	CCAGAAACAC	CAAGAACTOTY
										lud's:
17201	ATAMARTITE	CACCTUCCUC	: crecerries	COCTACCOGG	ATTICCGACKIA	ATTICKIARIA ARANTGENCE GTARARDORIO CARGOCCOOC CACOOCCTUA COGGERACAT	GTAGGAGGG	CATGGCCGGC	CACCOCCTOA	COCCCCARCAT
 	TATACCCCCCA			GCCACOCCC	TANGRETECT		TRITTACETTES CATECTECEE GTACCOGCES	GTACCOGCCG	GTGCCGGACT	<b>GCCCGCCGTA</b>
	奇				Sphil			:		-
17301	GCCHCCHCC	CACCACCGGC	300000000000000000000000000000000000000	GTCGCACCGT	CYN. ATMICINES	CCGCTATCCT	ACAGINICAT GCCCCTCCTT ATTCCACTOR TCGCCCCCCC GATTGCCGCC	ATTCCACTGA	100000000	GATTGGCCCC
	COCAGCACGC		בכפבבפבפבפ	CAGCGTGGCA	OCCITACGCGC		CHICATARIA CHICHAGAA TAAGSTIACT AGCORGGCG CTAACCGGG	TAAGGTGACT	AGCGGCGCCG	CTAACCCCC
17401	A ASSESSMENT OF THE PARTY OF TH		r GreenfigeAg	GCGCAGAGAC	ACTICATTANA	AACAAGTTAC		ATCITICADADA ATCADATA ANGTICTOGA	ANAGTETGGA	CTCTCACGCT
			A CCCCANCETC			TTGTTCAACG	TACACCTTTT	TAGTETTATE	TTTCAGACCT	GAGAGTGCGA
										Ecoliv
17501	مدعساسلاعتفلان	· SCIPACITAL	CONTRIBUTE PERABETATE PROTAGATG GANGACATCA ACTITISCENT TETBRECECTO COACACACAT GEGECEGFF CATAGGAAAC TIGEAMAATA	GAAGACATCA	ACTITIOCGIC	TUTGREETED	CCACACCCCT	CGCGCCCGFF	CATGGGAAAC	TRECAMENTA
* > 7 - 1	GCGMCCAGG	ACATTGATA	CICINATION CONTINUE AND AND AND A CONTINUE TO A DATE OF THE SECOND SECON	CITCTGTAGE	TGAMACGCAG	AGACCTAROXIC	GCTGTGCCGA	GCCCCCCCAA	GTACCCTTTTG	ACCGFFCFA?

Figure 15K

	ECORIV									
17601	TCGGCACCAG	CANTATGAGE	GGTGGCGCCT	TEAGGTGGG					ANGMACTATO	GCAGE ANGRE
! •	۶	CITTATACTCG	CCACCCCCCA	AGTEGACTEC (	ניטטטענענעטטטט	TY GCCGTAAT 1		MAGCTOCCAA	TICTIONIAL	
1011		Marketter	AGATICATIONS	GGATAAGTTO	AAACCACAAA	ATTRICTARIA A	AAACCTTCCTA	GATCCCCTCG	CCTCTGGCAT	することのころです
10//1	Character	month and the contract of the	TCTACGACTC		_	TAMAGGTTGT 1	TITICCACCAT	CTACCGGACC	CCACACCGTA	ATCGCCCCN:
	000000000000000000000000000000000000000				1 firefill					
17801	GTOGACCTOG	CCAACCAGG	ACTGCANAT	ANGALTAACA	Š		CCCTTAGAGG	AGCCTCCACC	CCCCGTCCA0	ACAGTGTCT.
	CACCTGGACC	GOTTAGTECES	TCACGITTITA	TTCTAATTGT	CATTACHANCT	ACKINGING T	TANCATCTIC	TCGGAGGTGG	CCGGCACCIC	TOTCACAGO
12901	CAGAGGGGG	TOCCGAAAAG	COTTCCCCCCC	CCGACAGGA	AGAMACTOTAS		TACACCANCC	TCCCTCGTAC	GAGGAGGCAC	TANAGCANT:
	GICTCCCCGC	ACCGCTTTTC	GCAGGCCCCG	GGCTGTCCCT	TETTTGAGAC	CACTGCGTTT /	ATCTOCTCGG	AGGGAGCATG	Crecrecord	ATTICGITIC
18001	CCTOCCCACC	ACCCOTCCCA	TCCCCCCAT	CCCTACCGGA	כנוגא_וואטטככ		COTAACGCTG	GACCTCCCTC	CCCCCCCGA	CACCCAGCA
	GCACGGGTGG	TOGGCAGGGT		AGCOCOGOTA . CCGATYGCCT	ב <b>אכניגנינ</b> 	TCCTUSTUSE (	GCATIGCGAC	CTGGACGGAG	Pvel	
							נטנטטטטטט	CCAGOXOTCC	OCCUNTOSTIO	COCCCOTAG
18101	AAACCTOTOC	-	GACCGCCGTT	CAACATEGGG			CCCCCCCCCCC	GOTCGCCAGG	COCTAGCAAC	GCCCIOGCATC
,	TTTOGACACG						AGEGEEGAFG	ATGCTTCTOA	TACCTAACGT	GICCITATOIN
18201	CCAGTOOCAA		ACACTGAACA		AGACCCCCAC		TEGEOGETICE	TACGRAGACT	ATCGATTGCA	CACKCATACA 1
•	GOTCACCUT	-			ACTOCOCCO		CCAAGATAGC	TACCCCTICG	ATGATGCCGC	
18301	TGICATOTAT	GCGTCCATGT			100000000		GGTTCTACCG	ATGGGGAAGC	TACTACGOCG	TCACCAGA: "
	ACABIACAIA					TECACITIES	CCGCGCCACC	GAGACOTACT	TCAGCCTGAA	TANCAAGTIIT
18401	CATGCACATC		ACCCC COCCA				GCCCCGGTGG	CTCTGCATGA	AGTEGGACTT	ATTOTTCAM
	GTACGTGTAG						CTRICETTICA	TCCCTGTGGA	CCCTGAGGAT	ACTCCCTACT
18501	AGAMACCCCA		-		MCC(AG) CI.CA	CCCANACTIC	GACGCCAAGT	AGGGACACCT	GCCACTCCTA	TCACCCATGA
	rerrroocer	_	_		-	A STATE OF THE PARTY OF THE PAR	CGTACTTICA	CATCCOCOOC	GTGCTGGACA	GOCCCCTA
10901	COTACAAOGC	_	_	_	TGTCGTGGAC	TACCOMOGE	GCATGAAACT	GTAGGCGCCG		
	OCATOTTCCG	_	_			THE CONTROL	TYTCAATEG	CATCAACCTO		TGAAATAAA
18701	TTTTAAGCCC	-		CACCCIVAACT		GREGETTAGG	AACCCTTACC	CTACTITEGAC	GATCACGAGA	ACTITATITIE:
		_	_	-		CCACCAAAAA	ACTEACGRAT	Trecented	<b>CCCFFATTCT</b>	CCTATAAATA
18801	CTAGAAGAAG	ACCACCATCA TO TACK	CAACCAAGAC	CITICATCAGG		CGTCGTTTTT	TRACTECATA	-	CCCAATAAGA	
	GARCITICA					GCCGATAAAA	CATTICANCE	TGAACCTCAA	ATACCACAAT	
18901	TTACANAGGA	GGGTATTCAA				CCCCTATITI	GENNAGTING	ACTIGGAGIT	TATCCTCTTA	-
	AATGTTTCCT	-				CAATGAAACC	ATTACAGE	TCATATGCAA		
19001	COMMENCATION		_			GTTACTTTCG	TACAATGCCA		•	_
		•	•	P GGANAGCTAG	NAACTICAACT	_	TITITICICA		_	-
19161	CCCOTTCCGT		_	_	THICKSTICA		MAMAGAGET			TACCACIAN
19201	ACTIVIACTO	-	A TTGTACAGTG	; ANGATGTAGA					TATTANGGANG	
1	TGAACTGAGG		r ANCATGICAC	: TTCTACATCT	ATATETTING	CKITCTGTCAG	TATAMGAAT	GTACKAGGTGA		

Figure 15L

19301	ACAACTAATO	GCCCAACAAT	CTATRICICAN					CTANTGTATT J	ACAACAGCAC ACAACAGCAC	GOSTANTATA:
	TCTTGATTAC	CCGGTTGTTA	CATACCCCTT	GTCCGRAJTA						
19401	COTOTICTOO	COCOCCANDO	ATCOMACTING	AATISCIIGITIG	TAGATTTGCA			_		TCCATTCGTV
	CCACAAGACC	OCCCOOPTICG	TAGGGTCAAC	TTACKINCAAC	ATCTAMACTO	TCTV;TCTTTG 1	TOTICTICONAN (	_		ACCITAACCIAL
19501	ATAGAACCAG	CTACTOTICS	ATCTCCAAATC	ACCUCATION	CACKTANTANT	CCAGATGITTA C	GANTTATTON !	ANTCATCGA 1		ANCTITICTAAA
	TATCFIGGIC		TACACCITING	TCCGACAACT		GGTCTACAAT (	CTTANTARCT .	TITAGTACCT	TGACTTKTAC .	TTGAA * :'I'T'F
19601			GICTGATTAA	TACAGAGACT	CTTACCANGG	TAMARICTAN 1	AACAGGTCAG	GAMATGGAT	-	TOCTACAGAA
1 1 1	AATGACGAAA	GGTGACCCTC	CACACTAATT	ATGTCTCTGA	GANTYATTICC	ATTENDENTE 1	PPOTCCAUTE (	CTTTTACCTA (	CCCTIMITICS	ACCATICIT
19701	TITICAGATA	AAANTGAAAT	AAGAGTTGGA	AATAATTTTG	CCATCERANT	CANTCTANAT (	GCCAACCTGT		CCHUTACTCC	AACATAGCO .
!	AAAAGTCTAT	THITACTITA	TTCTCAACCT	TTATTAMAC		GTTAGATTTA	COGFFCCACA	CCTCTTTAAA	CCACATGAGG	THETATICETY:
19801	TOTATITOCC	CCACAAGCTA	AAGTACAGTC	CTTCCAACGT	AAAAATTTCT	GATAACCCAA	ACACCTACCIA	-	AAGCGAGTOG	TOGCTCCC():
		_	TTCATGTCAG	GANGGITTGCA	APANATETE	CTATTGGGTT '	TOTOGATOCT	CATGTACTTO	TICOCTICACC	ACCGAGGGG
19901	OCTAGTGGAC	TOCTACATTA	ACCITIGGARC	ACCITAGICC	CTTCACTATA	TYXINCAACGT	CAACCCATTT			CCTGCGCTAC
	CGATCACCTG	ACCIATOTAAT	TEGAACCTCG	TOCCACCAGG	GANCTIGATAT	ACCTGTTGCA	GITGOGTANA	ricerectes	COTTACCACC	CCACCCGATO
20001	COCTCAATGE	TOCTOGGCAA	TOGICGCTAT	GIGCCCTTCC	ACATECACK	CCCTCAGAAG	TICTITICECA		certerical	CCGGCTCAT
1	GCGAGTTACA		_	CACCGGGAAGG	TGTANGTCCA	COCAGICTIC	NACHANCGGT	AATTITITICGA	GGANGAGGAC	GGCCCCAGTA
					Pstf					
20101	ACACTORACTOR	CHECANCIPIC	ACCAAGCATC	TTAACATOGT	TCTY TAGAGE	TCCCTAGGAA	ATGACCTAAG	COTTGACGGA	OCCAGCATTA	AGTITICATA:
	TOTOGOATOCT		-	AATTGTACCA	AGACCTICTICG	AGGATCCTT	TACTGGATTC	CCAACTGCCT	CCCTCCTAAT	TCAMCTAIN
20201	CATTIGGG	_	-	GCCCCACAAC	ACCIOCITOCA	CGCTTGAGGC	CATCCTTAGA	AACGACACCA	ACCACCACTC	CITTANCO!
	GTAAACGGM	_			TOXXCIRCAGGT	GCGAACTCCG	GTACGAATCT	TRICTOTOGE	TECTOOTCAG	GANNITOC'I :
20301	TATCICTO	_	GCTCTACCCT	ATACCCGCCA	NCCTACCAA	CONGCCCATA	TCCATCCCCT	CCCGCAACTG	OCCOCCTTTC	COCOROCACIONS
	ATAGAGAGGC		_	TATCGGCGGT	TRECATEOUT	GCACGGGTAT	ACCTACCCCA	GOCCUTTGAC	CCGCCGAAAG	GCGCCGACTT:
20401	CCTTCACGCG	-	- MORINANCEC	CATCACTGGG	CTCCGGCTAC	GACCCTTATT	ACACCTACTC	TOCCICIATA	CCCTACCTAG	ATGGAACTT
! ! !	GUANGTOCOC	COMMITTETON	TTCCTTTGGG	GTACTGACCC	GAGCCCGATT	CTGGGAATAA	TCTCCATGAG	ACCGAGATAT	GGGATGGATC	TACCTTCX !/ A
20501	TTACCTCANC	CACACCTITA	AGANGGTGGC	CATTACCTTT	CACTCTTCTT	PCARICTRACC	TECCANTEAC	COCCIDENTA	CCCCCAACGA	GITTICANATIC
	AATCGAGTTG	CHCTCCAAAT	TETTICCACCG	GTAATGGAAA	CTCAGAGAC	ACTECACEDO	ACCUITACTO	CCCCACCAAT	COCCUTICAT	CAMCTITIA
20601	AAGCGCTCAG	3 TTGACGGGGA	GOCTTACAAC	GTTGCCCAGT	CITAACATGAC	CANAGACITAG	TICCTURETAC	ANATOCTAGE	Thactatanc	ATTK# XCTACK
	Treacanate	: AACTGCCCCT	r cccaanerie	CAACGGGTCA	CATTIGTACTG	GTTTCTGACC	MGGACCATO	TTTACCATCG	ATTGATATTG	TWCCGATCK
20701	AGGGCTTCTA	A TATECCAGAG	3 ACCTACAAGG	ACCOCATOTA	crecriterity	AGAMCITICO	AGCCCATGAG	CCGTCAGGTG	GTGGATGATA	CTANATACAA
	TCCCGAAGAT	F ATAGRAGICTE	: TCGATGTTCC	TYSCGTACAT	GACCAAGAAA	TCTTTCAGG	TCGGGTACTC	GCCACTCCAC	CACCTACTAT	GATTTATGTT
20801	GCACTACCAA	A CAGGTGGGCA	A TECTACACCA	ACACAACAAC	-	TTGGCTMCT	TOCKCCCACC	ATCCCCCAAG	GACAGGCCTA	CCCTRACTAM.
	CCTGATGGT	r stecacees	r AGGATGTGGT	TOTOTHOPIO	AGACCTAAAC	AACCGATGGA	ACGCAGGTGG	TACGCGCTTC	CTOTCCGGAT	GOGNECATING
							Pvid			
20901	TTCCCCTATC		3 CAAGACCGC				TYSCIATCOCA	CCCTTTGGCG	CATCCCATTC	TCCAGTAACT
	AACCCCATAG	G OCCUNTATIC	C GITCTGGCCT	CAACTESTEGT	AATGGGTLTT	TTTCAMGA	ACGCTAGCGT	GEMANTEGE	GIAGGEI MAG	ACTION 100

Figure ISM

									distriction	3
21001	TTATGTCCAT	CCCCCCACTC	ACAGACCTAG TRETCTAGACC	GCCANAACCT	TCTCTACGIC	AACTCCCCCC	ACCCCCTINGA	CATCACTETITE	GAGGTUGATE	CCATOCACGA
	William Charles					9.30000000	ر در	ATTORNACION	ACT PRODUCTION OF THE PROPERTY	ر لاردندسلما
21101	OCCCACCCIT	CTTTATGTTT	TOTAL		נישניבניייניייי	ACCAS COM	יייייייייייייייייייייייייייייייייייייי	AICGMANCE	ופושררומופ	
	COGOTOGOAA	GAANTACAAA	ACAMCTITCA	GAMCTGCAC	CAGGGACACAG	TUGICIOCOT	GREGEEGENG	TACCTITICGC	AC/ TYCACCC	GICCOCCAV :
										light.
21201	Tristitut	ACTURACAAC	ATAAAGAAGC	AAGCAACATC	AACAACAGCT	GCCCCATGG	GCTCCAGTGA	GCAGGAACTG	AAAGCCATTG	TCANAGATOT
	Adecedent	reconstra	TATTICTICG	TICGTINGTAG	TIGITIGICIA	COUCOGIACE	CGARTCACT	CGTCCTTGAC	TTTCOGTANC	Agrenctaga
10110	Transferred	CCATATETT	TREECACETA	TRIACAAGCGC	TTTCCAGGCT	THEFTETCE	ACACAAGCTC	GCCTOCOCCA	TAGTCANTAC	COCCUSCION»
	ACCAACACC	GGTATAAAAA	ACCCUTGGAT	ACTOTTCGCG	AAAGGTCCCA	AACAAAGAGG	TGTGTTCGAG	CGGACGCGGT	ATCAGITATE	CCGGCCAGIX
21401	GAGACTOGGG	GCOTACACTO	GATGCCCTTT	GCCTCGAACC	CGCACTCAAA	MCATOCTAC	CTCTTTGAGC	CCTTROGCTT	<b>TTCTGACCAG</b>	COACTCAAGC
1	CTCTGACCCC	COCATICTICAC	CTACCGGAAA	COCACCITION	GCGTCACTFF	TIVITACCATG	GAGAMCTCG	GGAAACCGAA	AAGACTOGTC	<b>GCTGACTTC</b>
21501	AGGITTACCA	GTTTGAGTAC	GAGTEACTEC	TOCGCCCTAG	CGCCATTCCT	TCTTCCCCCG	ACCOCTGTAT	AACOCTOGAA	ANGTECACCC	AAAGCGTAC .
	TCCAAATGGT	CANACTCATO	CTCAGTGAGG	ACCCCCCCATC	GCGGTAACGA	AGAAGGGGGC	TGGCGACATA	TTGCGACCTT	Trcaggrada	TTTCGCATGF
21601	GOOCCCAAC	TCGGCCGCCT	GTGGACTATT	CTCCTCCATG	TTTCTCCACG	CCTTTGCCAA	CTGGCCCCAA	ACTCCCATGG	ATCACANCCC	CACCATGAA
	CCCCGGGTTG	ACCCGGCGGA	CACCTGATAA	GACGACGTAC	AAAGAGGTGC	GCAAACGGTT	GACCGGGGTT	TCACOGTACC	TAGTOTTOOO	OTGGTACTT .
		Kpm			No.		•			
21701	CTTATTACCO	OCCTACCCAA	CICCATGCTC	AACAGTOCCC	ACCINCACK	CACCCTGCGT	COCANCCAGO	AACAGCTCTA	CAGCITICCTO	_
	GAATAATGGC	CCCATOGOTT	GAGGTACGAG	TTGTCAGGG	TCCATCTCGG	GTCCCACCCA	<b>GCGPTGGTCC</b>	TYGTCGAGAT	GTCGAAGGAC	CTCGCGGTG
21801	COCCTACT	CCCCAGCCAC	AGTECECAGA	TTAGGAGCGC	CACTICITIT	TUTCACTTOR	ANAACATGTA	AAAATAATOT	ACTAGAGACA	CTTTCAATAA
	GCGGGATGAA	gocorcogra	TCACGCGTCT	AATCCTCGCG	GTGAAGAAA	ACAGTGAGT	TITIGIACAT	TTTTATTACA	TOATCICTOT	GAAAGTT?AT"!"
21901	ACCEANATE	TTTTATTOT	ACACTCTCGG	GTGATTATTT	ACCCCCACCC	THECCGRETO	COCCUTTAN	ANATCANGO	GOTTICTOCCO	COCATCOCTA
	TCCOLTTACO	AAAATAAACA	TGTGAGAGCC	CACTAATAAA	reconcises	NACGCCAGAC	GCGCCAAATT	TITAGITICC	CCANGACOGC	GCGTAGCGAT
22001	TEXTOCCACTO	GCAGGGGACAC	GTTGCGATAC	TOGTOTITAG	TYCTCCACTT	AAACTCAGGC	ACAACCATCC	GCGGCAGCTC	OCTCAACTTT	TCACTCCACA
	ACCCCGTGAC	CONCENSIO	CAACGCTATG	ACCACAMATE	ACCENGENCE	TTTGAGTCCG	TOTTIGGTAGG	CCCCCTCCAG	CCACTTCANA	AGTCAGGTGT
					EcoffV					
22101	GGCTGCGCAC	CATCACCAAC	GCCTTTACCA	GGTCGGCCC	CCATATCTTG	AAGTCGCAGT	TORGGCCTCC	OCCURAÇÃO	COCCAOTTOC	_
	CCGACGCGTG	GTACTOGTTG		בכעפניבנפנפ	CCTATAGAAC	TYCAGCOTCA	ACCCCCCCAGG	COCCACCCCC	GCGCTCAACG	CTATISTICAL:
22201	GTTGCAGCAC	TOGAACACTA	TCAGCGCCCG	GTGGTGCACG	CTYCCCAGCA	COCTOTOR	CCANGATCAGA	TCCGCGTCCA	CONCINCOC	GTTCCTCAGG
1 2 3 3	CAACGTCGTG	ACCTIGICAT		CACCACGTGC	GACCAGTCGT	GCCYCANCAG	CCTCTAGTCT	AGGCGCAGGT	CCAGGAGGCG	CANCGAGTICC
10166	Characterina	TCAACIFICAS:	TAGCTGCCTT	CCCANANAGG	מטמנונונומטנונ	ACCUTTTCAC	TRECACTOR	ACCOTAGTOO	CATCANANGG	TOACCOTOCC
	COCTIOCCTC	AGTTGAMACC			מפכטכעכעכע	TCCGAMCTC	AACGTGAGCG	TOGCATICACC	GTAGTTTCC	ACTROCACOG
22401	COOLCTOOOC	GTTAGGATAC	AGCGCCTGCA	TAMARGEETT	GATCTGCTTA	AAAGCCACCT	CAGCCTTTGC	GCCTTCAGAG	AAGAACATGC	CGCNAGACTT
)	OCCAGACCC	CAATCCTATG	TCCCGGACGT	ATTTTCCCAA	CTAGACGAAT	THEGGINGA	CTCGGAAACO	COCIMACI I: TIC	TICTIONACG	·
			Stil				DO!			
22501	GCCGGNAAAC	C TGATTCGCCC			CACCACCTAG			ACCACATTIC	GCCCCACCG	GITCTICACG
•	CGCCTTTTG	G ACTAACCGGC	CTGTCCGGCG		CAGCACOTUC GTCGTGGAAC	CCACCACA	CCTCTAGACO	TOGETHANGE	درهمواهد	

Figur 15N

22601	ATCTTOGCCT	TCCTAGACTC	CTCCTTCACC	CHOCETICANG GONDALINGG CONTINUACIT GARANACITCS CROMISAND GCANANGGA			ATTICAATCA C	COTOCTCCTT A	ATTTATCAPA TAAATAGTAT	ATGCTTCCGT TACGNAGGCA
										11-11
22701		AACCHICATOR	TYCATTYTY	LUCTOCCCTG	נאגניאניאט	מנפנאמככנם ב	TCCCCTCCTC 1	ATCCTTCTAG C	<b>GTCACCTC*N</b>	CNANCGACTG
10.77	CATCTGTGAA						אככניפאטינאכ י	TACGAACATC C	CACTGGAGAC	GTTV:CTC:W.
	P311									الليكينينية. ا
22801	CAGGTACGCC	TOCAGGAATC	<b>GCCCCATCAT</b>	CCTCACAAAG	CACAMOTAGE					
	GICCATGCGG	ACGICCTTAG	COCKCTAGTA	GCAGTGTTTC	CARANCAACG	ACCACITECTA C				GITCCAGAAC
10066	CATACCATO	CCAGAGCTTC	CACTTGGTCA	CCCACTAGTT	TRANGITUDE	CTTTAGATEG 1	TTATCCACGT		CATCAGCGCG	CIRCIACAGOCT
	GTATGCCGGC	GGTCTCGAAG			ACTICAAGCG	GAAATCTAOC /	AATAGGTGCA	CCATGAACAG	GTAGTCGCGC	acoconcor 1
			Fwel							. ;
23001	CCATCCCCTT	CTCCCACGCA			CGGGTTCATE:				refrector	CCTCTTGCG"
	OGTACOCCAA	GAGGGTGCGT	CTCTCCTAGE	COTGTGAGTC	CCCAAGTAG				RIGARIACAMA	שיייייייייייייייייייייייייייייייייייייי
23101	CCCCATACCA	COCOCCACTO	<b>GCTCGTCTTC</b>	ATTICACACICIC	recoentates		THRECATES		CCOGLGGGIT	GCTGAAACOX:
	GCCGTATOGT		CCAGCAGAAG	TANGTEGGEG	OCCINONCACO	CONTECHED	MACGGTACG		OCCACCCAA	COACTITION
10212	ACCATETOTA	GCGCCACATC	TRETETITET	TCCTCKKTCT	CCACCATTAC	CICIOSTGAT			ACANGGGCGC	PICTURE
	TCCTAAACAT		AAGAGAAAGA	ACCACACACA	GGTGCTANTG	CAGACCACTA	CCCCCCCCCA	GCCCGAACCC	TCTTCCCGCG	AAGAAAAAGA
10116			Tecoecece	ACCITCUATED	CCCCCCCCTC	CONTINCECC	GCACCAGCGC	<b>GICTROTOAT</b>	GAGICTICCE	CONCINCORA
	AGAACCCGCG		AGGCGGCGGC	TCCAGCTACC	<b>GCCCCCCAC</b>	CCACACGCGC	corcorcoco		CTCAGAAGGA	GCAGGGGGGT
21401	CTCCATACGC		<b>BCTTTTTTGG</b>	000000000	CCACACCCCC	GCCACCCCGGA	CURRACTOR		TOCTTCCCCC	Accrecein.
	CAGCTATGCG			CCCGCGGGGCC	CCTCCGCCGC	CACTGCCCCT	OCCCC:1GC1G		ACCAACCCCC	TGCAGCGC
215.01	(ar Account			COCTOCTCCT	CTTCCCGACT	GRECATITICS	TICHGUTATA		GATCATGGAG	TCAGTCGAGA
1			CCACCANAGC		GANDOCCTGA	CCCCTAAAGG	ANGAGGATAT	cconcinning	CTAGTACCTC	AGTCAGCTC"
23601			CCCTCTGAGT	TCGCCACCAC	CGCCTCCACC	GATGCCGCCA	ACGCCCCTAC	CACCTTCCCC	GTCGAGGCAC	CCCCGCTTGA
		C GGATTGGCGG		ACCCCTCCTC	GCGATACATIVAT	CTACGGCGGT	TCCCCCCATG	OTOGAAGGGG	CAGCTCCCTG	GGGGGGAACT
21701	COACOCACO	A GRICATTATICO	ACCAGGACCC	ACCITITIETA	AGCCAAGACG	ACGAGGACCG	CTCAGTACCA		AAAAGCANGA	CCAGGACAN'
1	CCTCCTCCTT			TCCANAACAT	1CGCF1CTGC	TOCTOCTOGG	GAGTCATOOT	TOTAL	THE STACE	THINCOTACT CONCERCIANS
										***************************************
23801	CCAGAGGCAA	A ACGAGGAACA	AGTETAGGCGG	GOCCACGAM	<b>GOCATORICA</b>		GTOOGAGACT		GAAGCATCTG	
	CONCINCION			CCCCTOCTIT	CCGTACCGCT	GATGGATCTA	CACCCTCTGC	TRCACCACAA	CTTCGTAGAC	
23901	CORCEANTA	_	-	GCACCCATGT	RECECTEGACE	ATAGCOGATE TCAGCCTTGC	TCAGCCTTGC	CTACGAACGC	CACCTATTCT	
	CGCGGTAATA		-	CONCOCTACA	CCANAGAGGGG	TATCGCCTAC	NGTCGGNCO	GATGCTTGCG	GTGGATAAGA	
74001			ACCACACATO	CCAGCCCAAC	CCCCCCCCCACA	ACTICTACCC	CUTATTRICC	GTGCCAGAGG	TGCTTGCCAC	
70047	TGGGGGTTT					TCAAGATGGG	GCATAAACGG	CACGGTCTCC	ACGAACGOTG	GATACHCTA:
24101	THEFT	A ACTOCARGAT	ACCCCTATCC	TRECESTIGEEN	ACCOUNTER	TRENGTISCEN ACCOUNTICG AGGGACIAG CAGCTOSCCT TREINGLAGGG	CAGCTGGCCT	TREFREENCE	CCCTCTCATA	
! !			TOGOGATAGG	ACCCCACGGT	TOGGGTCTCCGC	TEGECTOTIE	GTCGACCGGA	ACGCCGTCCC	GCGACAGTAT	GGACTATAG

Figure 150

# PMRRAdSgag MFR682

24201	CCTCGCTCAA	CGAAGTCCCA	MAMATETTES TTTTAGAAAC Xhal	ARSGRETTING TCCCAGAAACC	ACGCCACCAG TGCGCTGCTC	AAGITGAGGTEG TTGGGGGGGGGG	CAAACCICTCT	COTTOTCCTT	AACAGCGAAA	ATGANAGTCA TACTTTCAGT
24301	CICTODAGTO	THEOTOGRAC AACCACCTTO	<b>&gt;</b> -	CAACGCGCGCG	CTAGCCGTAC	TANAACTICAG	CATCTACATC	ACCCACTITIO (	CCTACCCOOC	ACTIVACETA TOAKTIGGAT
24401	CCCCCCAAGG	TCATGAGCAC AGTACTCGTG	AGTEATGAGT TEAGTACTEA		Tracercetric Acercerce					GAGGAGGG""
24501	TACCCGCAGT	TOCCGACGAG	7	GCTORCTTCA	AACGCGCGCGCTC	CCTCCCGACT	TRIGARGAGED	ACCCAAACTA	ATGATGGCCG TACTACCGGC	CAGINICITORIT
24601	TACCGTGGAG		CITICAGTGCA TGCAGCOGTT GAACTCACGF ACGTCGCCAA Bgill	CTTTGCTMAC GRANCGACTG	CCGGAGATGC	AGCCCAAACCT	AGACCIAAACA TCTCCTTTGT	TTGCACTACA (	CCTTTCGACA	GOGCTACGTA
24701	COCCAGGCCT	COTTCTAGAG	CAACGTGGAG	CTCTGCAACC GAGACOTTGG	TGGTCTCCTA ACCAGAGGAT	CCTTGGAATT GGAACCTTAA	TTGCACGAAA AACGTGCTTT	ACCOCCTTOG TOGCOGAACC	OCNANACOTO COTITTICCAC	CTTCATTCCA
24801	CGCTCAAOOG		COAGGCGCG CGCGACTACG GCTCCGCGCG GCGCTGATGC	TCCGCGACTG	CCTTTACTTA	TTTCTATGCT ACACCTOGCA AMGATACGA TSTXRACCGT	ACACCTGGCA TYTYXACCGT	GACGGCCATG GGCGFTFGGC CTGCCGGTAC CCGCAAACCG		AGCAGTGCT1' TCGTCACGAA
24901	GGAGGAGTGC	AACCTCAAGG	AGETGEAGAA	ACTGCTAAAG TGACGATTTC	CNAAACTTGA GTTTTGAACT		CHACOGCCITIC CHACCGGANG		CCCTOGCCCC	OCACCTOSICY: CGTGGACCQV:
25001	GACATCATIF	-	CCTCCTTAAA	ACCCT/3CAAC TGGGACGT/1G	ARCENGACGG TCCCAGACGG	AGACTTCACC TCTGAAGTGG	ACTCANAGCA TCAGITTCGT		CTTTAGGAAC	TTTATCCTM.
25101	AGCGCTCAGG TCGCGAGTCC Pell	ANTCTTGCCC	<b>GCCACCTGCT</b> CGCTGGACGA	GTGCACTTCC	TACCCACTT	GACGGGTAAT	AGTACCGCGA TCATGGCGCT	ANGCCCNCCO TACOGGAGGC	CCCTTTOO	GCCACTCCTA CGCTCACGA'F
25201	CCTTCTGCAG	CCTTCTGCNG CTAGCCAACT GOANGACGTC GATCGGTTGA	ACCTTOCCTA TGGAACGGAT	CCACTCTGAC GGTGAGACTG	ataategaag Tattacctte	ACGICACTOCC ACTOCCAGAT TOCACTCCC ACTOCCAGAT Mani		CTOBAGTOTC GACCTCACAG Pstl	ACTIGACAGCGAC TGACAGCGAC	CAACCTATRIC
25301	ACCCCGCACC	OCTCCCTOOT CGAGGGACCA	TTGCAATTCG AACGTTAAGC	CACCTCCTTA GTCGACGAAT	ACCAMAGICA TGCTTTCAGT	AATTATCGGT ACCTTTGAGC TTAATAGCCA TGGAAACTCG		TECAGGETICE ACOTECEAGE	CTCGCCTGAC	GANNAGTECH CTTTTCAGGC
25401	CGGCTCCGGG	GTTGANACTC CAACTTTGAG	ACTCCGGGGC TGAGGCCCCG	TOTOGACOTO ACACCTGCAG	GGCTTACCTT CCGAATGGAA	CCCAAATTITA	TACCTGAGGA	CTACCACGCC	CACGAGATTA	GOTTCTACGA
25501	AGACCAATCC TCTGGTTAGG	COCCCOCCTA	ATGCGGAGCT TACGCCTCGA	TACCRCCTRC ATGGCGGACS	GTCATTACCC	AGEGGGETGEA TCCCGGTTGEA	TCTTGGCCAA ACAACCGGTT	TTGCMGCCA	TCAACAAAGC AGTTGTTTTCG	CCCCCMGM GCCX:TTCT(
25601	TTTCTGCTAC	GAMAGGRACG	GGGGGTTTTAC CCCCCAAATG	TTGACCCCC ANCCTGGGGG	AGTCCGGCGA TCAGCCCCCT	GCACCTCAAC CCTCGAGTTG	CCAATCCCCC	GCCCCCCCCCA	GCCCTATCAG	CARCARCCO !

Figure 15P

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25701	<b>GGCCCTTGC</b>	Treceagn	CCCACCCANA	AAGAACCTCC	N.X.Train	שניאנינינאנים י			CAGTCAGCCA	CAGGACACTET
٠	CCCGGGAACG	AAGGGTCCTA	CCGTGGGFTT	TICITICGACG	<b>TYTENCYCICA</b>	GTGC	CTGCTCCTCC	TTATGACCCT	GTCACTCCGT	CHICCHCCA .
						I levelill				
25801	TOGACGACCA	CCACCACCAC	ATCATCACANG	ACTORBOARAG	מבדאהאההאה	E	AGGICGAAGA	GETETCAGAC	GAMACACCGT	CACCETTOOK .
	ACCTGCTCCT	CCTCCTCCTO	TACTACCTIC	TGACCCTCTC	GGATCTGCTK:	CTTCGAAGGC	TCCAGGTTCT	CCACAGACTG	CTTTGFGGCA	GTCCCCAGC(A)
25901	CGCATTCCCC	TCGCCGGCGC	CCCAGNANTC	GCCAACCGGF	TECAGEATES	CTACAACCTC	CGCTCCTCAG	00000000000	CACTGCCCGT	TOTACOGACCC
	GCCTAAGGGG	AGCGGCCGCG	GOTTETTAG	CCCTTGGCCA	ACCITICATACE	GATYTTYGGAG	CCGAGGAGTC	כעכמסכמפכנ	GTCACGGGCA	ACCOCTOGG
26001	AACCGTAGAT	GGGACACCAC	TGGAACCAGG	GCCCGTAAGT	CCMACAGC	CCCCCCCTTA	GCCCANGAGC	NACANCAGOG	CCAAGGCTAC	COCICATOO!
	TTOOCATCTA	CCCTGTGGTG	ACCITIGGICC	COCCATTCA	GGTTCGTCGG	CRRCARCANT	CGGGTTCTCG	TIGHTGICGC	GGTTCCGATG	OCGAGTACCU;
26101	GCOCOCACAA	GAACGCCATA	grtectiact	TOCANGACTO	TEXTOREGENAC	ATCTCCTTCG	CCCGCCGCTT	TUTTETAC	CATCACGGCG	المحكالة الملائدات
	COCCONOTE	CFFCCCCTAT	CHACGAACGA	ACCITICAC	ACCCCCGTTG	TAGAGGAAGC	GGGCGCCGAA	ACANGAGATO	GTAGTOCCGC	ACCITICIDAGGY.
26201	CCGFAACATC	CHGCATTACT	ACCOPCATET	CTACAGCCCA	TACTECACES	CCCCCCACACACACACACACACACACACACACACACACAC	CAGGNACAGG	AGCGCCACA	CAGAAGCAAA	GREGACEGGA
	GGCATTGTAG	GACCTANTGA	TGCCAGTAGA	GATGTCGGGT	ATGACGTGCC	COCCORCOCC	Greenste	reaccount	Greencom.	concrescen
26301	TAGCAAGACT	CTGACAAAGC	CCAAGAAATC	CACAGCGGCG	GCARCARCAR	GAGGAGAAGC	GETOCGTETO	GCGCCCAACG	AACCCGTATC	GACCCGCGAG
	ATCOFTCTGA	GACTIGITATION	GGTTCTTTAG	Grencecese	corcorcorc	CICCICCICG	CGACGCAGAC	cocosomoc	TTGGGCATAG	CTGGGCGCTC
26401	CTTAGAACA	GUATTITICC	CACTCTRITAT	CCTATATTE	AACAGAGCAG	GROCCANGAA	CANGACCTOA	AAATAAAAA	CAGOTETETO	CCATCCCTCA
	GAATCTTTGT	_	_	CCATATAMG	TREMETORITE	CCCGGTTCTT	GITCTCGACT	TETATEMENT	GICCAGAGAC	CCTACCCACT
26501	CCCGCAGCTG	CCTOTATCAC	ANAGCCAAG	ATCAGCITICG	GCGCACGCTG	GNACACGCGG	ABBOTOTOTA	CACTAAATAC	TOCOCCIACION	CTCTTAAGGA
	GOOCGTCGAC	GCACATAGTO	trincectine	TAGTCGAAGC	CCCCTTCCCAC	CTICIOCOCC	TCCGAGAGAA	GICATHTATO	ACCCCCCACT	GAGAATTCC .
26601	CTAOPTICGC	GCCCTTRCTC	AAATTTAACC	CCCANAACTA	CONCARCINCO	ARCORCCACA	CCCGGCGCCA	GCACCTGTTG	TCAGCGCCAT	TATKTAGCAAG
	GATCAAAGCG	COOCAMAGAG	TITAMATICG	CGCTTTTGAT	GCAGTAGAGG	reaccastat	GOCCICCOCT	CCTCCACAAC	AGTEGEGGGTA	ATACTCOTTIC
26701	GALATTICCCA	COCCCTACAT	OTCCAGTTAC	CACCCACANA	TOGGACTICAC	<b>GOCTGGAGCT</b>	GCCCAAGACT	ACTCAACCCG	AATAAACTAC	ATGANCGCOS
	CTTTAAGGGT	OCCOGNICIA	CACCTCAATG	Gregoreter	ACCCTGAACG	CCGACCTCGA	CCCCTTCTCA	TCAGTTGGGC	Traittigate	TACTCOCOCC
		ECORY			ш <b>3</b>	Colli				
26801	GACCCCACAT	٠, ٠	GTCAACGGAA	TACOCOCCCA	CCCIANACCCA	CCCAAACCCA ATTUTCTCTCG	MACAGGCGGC	TATTACCACC	ACACCTCOPA	ATAACCITIAA
	CFGGGGTGTA	CTATAGGGCC	CAGTIGCCTT	ATGCGCGGGT	CRACITIFICACE	TAAGAGGACC	THETECGECG	ATANTGGTGG	TOTOGAGCAT	TATTOGAAT'
26901	TCCCCGFAGF	TOCCCCCTG	CCCTRISTIGIA	CCAGGAMAGT	CCCCCTCCCA	CCACTGTGGT	ACTITCCCAGA	GACGCCCAGG	CCGAAGTTCA	GATKINCTANK
	AGGGGCATCA	ACCOMICGAC	GGGACCACAT	GGTCCTTTCA	CATOCCIACOCT	GOTTGACACCA	TCAAGGGTCT	CIGCOGGICC	GGCTTCAAGT	CTACTGATTO
27001	TCAGOGGCGC	AGCTTGCGGG	COSCIPINCE	CACAGOGING	CONTRACCOCA	GCAGGGTATA	ACTICACCTICA	CANTCAGAGG	GCGAGGTATT	CAGCTCAACT
	AGTCCCCCCCC	TEGNACOCCC	GCCGANAGCA	OTGICCCACG	CCARCGGIACC	CCTCCCATAT	TCAGTGGACT	GITAGICICC	CGCTCCATA	GICGAGTTON.
27101	ACGAGICGGF	GAGCTCCTCG	CITICOTOTO	GTCCGGACGG	GACATTICAG	ATCGGCGGCG	CCCCCCCCCCC	TICATTICACG	CCTCGTCAGG	CANTCCTAAC
	TEXTENDECEA	CTCGAGGAGC	GAACCAGAGG	CAGCCTGCC	CTCTAAAGIC	TAGCCACCAC	GCCCGCCGAG	AAGTAAGTGC	GGAGCAGTCC	GTTACCATTIC
	Pati					٠			,	
27201	TOTOCAGACC	: repreciens	ACCCCCCCCC	TOGAGOCATT	GGAACTCTGC	AATTTATTGA	GGAGTTTGTO	CCATCOGICT	ACTITAACCC	
	AGACOTOTOG	I AGCAGGAGAC	TCCGCGCGAG	ACCTCCGTAA	CCTTGAGACT	TTAMATAACT	CCTCANACAC	GGTAGCCAGA	TCANATTGGG	GNACACCCT

Figure 1561

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00 4. 00			CHATACTAAT CATAAGAF*. GCCTGCTN: 3 CGGACGACAC	•		A CACCAGAGA A TACANARGA T ATGITTACE F A ARCHANTE A T TTGITTAN F G ATATGCAGAF
AAGTGGAGAG TTCACCTCTC GGGCTCCTAG TAGTTGAGC ATCAACTCGC	TAATAAATAC ATTATTTATG TACTTTTAAC ATGAAAATTG	AACACCACCC TTGTGGTGGG TCAATAACTC AGTTATTGAG	ACTICTACGGG TGAGATGCCC AAGGCTCGCC TTCCGAGCGG	TCACCCTTGC AGTGGGAACG TATAAATGC		AGINIMAGIT TCATATTCAA CTATATTAAA GATATAATTT CTGCTTGCAA GACGAACOTT TCTATGTGGG
ACCOGOODED TOTAL ACTION ACTION ACTION ACTION ACTION ACCOUNTY ACCOGOODED TOTAL ACCOGOODED TOTAL ACTION ACTION ACCOUNTY ACCOGOODED TOTAL ACCOUNTY ACC	GACGACTCAT CACGACTCAT CCTTACCTOG GGAATGCACC	CATCAGAAAA GTAGTCTTTT CGGACAGACC GCCTGTCTGG	ANTICAGOCA TTAAGTTCGT TTCTCTGCCT AAGAGACGGA		•	ATCHGETANC TACTCGITTG GTACCCTACT CATGGGATGA CGTATACTCG CGAANTIGAG AACAATTGAG TTGTTANCTG
GACGASCTACG CTRACTENTIC ACTITICACA TCAAAACGAT GTTTACCCAG CAAATGGGTC	TGCCATCTCT ACGCTAGAGA CCAAGGCGAA GGTTCCGCTT	TEAGCTACTC AGTCGATGAG AGACTTTTTC TCTGAAAAAG	GTTTATGAAC CAAATACTTG CAAATACTGCG TAATGATTGCG	-		TACCATGTAC  ATGGTACATG  CGAAACCAGA  ACCACTAACT  TGGTCATTGA  TGGTCATTGA  CATTCCCCTG
CCTCAGCCTC GACTYCGGTG CTGAGGCCAC TGATTGGGGA ACTAAGCCT	AGATCTTTGT TCTAGAAACA CCCAAGCAAA GOOTTCGTTT	CTCTCCGAGC GAGAGGCTCG ACCGTAAACC TCGCATATTGG	CTACTGTCOG GATGACACCC CTTTATTCTT	AGANGTIGAN TCTACTAATC	GCGTCGACTT TATGCTATTT ATACGATAAA	TGTGCGACNT ACACGCTGTA TACAGTGCTC ATGTCACGAG AGCTAATTACAG TGCATTACAG ACGAGTTATG
ACCEPTANA CTTMFCCCT GANV FREE GCCCTTMGC CCGCCATCG	GATTACATCA CTAATGTAGT TCTTCACCG	ACCIACIACIANC TOCTCTCTTG CTACCGCCTG GATGGCGGAC	AAAAGCGCAG TTTCCGCGTC TTGTGATTCT	TCGCCACCCA AGCGCTGGGT	TACANTETAN GTAITSTT CATACGACAA	TITIATGANA ANANTACTITE CTATACTANT GATACTATACAA ATTCAATTITC CCTCATTITC CCAGTANAGG
CCTAACTTYS AGGATTGAAAC GCCACAAGTG CGGGGTTK:AC GGGGAACCTT CCCTCTCGAA	CCTAACCCTG GGATTGGAC AACGCCACCG TTGCGGTGGC	CHCACTCAGA TREACCACACA ACOTOCACACA	GTATTAGGCC CATAATCCGG ATTCTCTGTC	AACGCTTCCG TTGCGACCCC	GGTCGGACAT AAATTGGCAA TTTAACGGTT	TACTTTTCCA ATCANAGGT ACGACGTGAC ACGACGTGAC GAATTAATTAC GAATTAAATG
TCANTTAINTY  GACTGACACC  GTGACACC  TTACCGCCC  AATGCCCCC  AATGCCCCC  AATGCCCCCC  AATGCCCCCC  AATGCCCCCC  AATGCCCCCC  AATGCCCCCCC  AATGCCCCCCC  AATGCCCCCCC  AATGCCCCCCC  AATGCCCCCCC  AATGCCCCCCCC  AATGCCCCCCCC  AATGCCCCCCCC  AATGCCCCCCCCC  AATGCCCCCCCCC  AATGCCCCCCCCC  AATGCCCCCCCCC  AATGCCCCCCCCCC	THECAACTET AACGTREACA CCATCCTGTA GGTNGGACAT	AACCCAGACG TTGGGTCTGC CACCGGCGGC	AACCCTTAGG TTGGGAATCC GGTTGGGGTT	CCAACCCCAA CAGCTTTTTA GTCGAAAAAT	AAATTCCTC GG CACAAAACA AA GTGTTTTTTT TT	CTITTATGTA GAMATYCAT TGGCACTITC ACCGTGAMAG MACAMATGC TTCTTACG GAMTAGGATT
ACTATECOGA TGATAGOCCT TGTGGACCAG GOCGACGGGC	TCACTGIGAT AGTGACACTA GCTCCTATCG CGAGGATAGC	CAACAGTITIC GITGTCAAAG ACGAGTGCGT TGCTCACGCA	GAGCTFAGAA CTCGAATCTT Xbal CFAGAATCGG		TETTCCACCT GCTTATTCGC CGAATAAGGG	AGTEATAMA TEAGTAATTTT TGGAANACHC ACCETTTGTG TATTGAGGA ATAACTCCTT ATTATAATTAAT
CCTCCCGGCC GGAGGGCCGG TGCCCTGAA ACGCGGACTT CCCCGGCGACTT GGGGGCGCGGGGGGGGGG	CCCTGTGTTC GGGACACAAG ATATACTGGG TATATGACCC	CTGTGATTTA GACACTAAAT CCGGGAACGT GCCCTTGCA	ANCAGGAGGT TTGTCCTCCA	AGTCCAAAGA TOCACATTTG ACGTGTAAAC Kori	GOTACCACCC CCATGGTGGG ATGAAAAGCT TACTTTTCGA	CCAGGOTANA GGTCCCATTT CAAATTGTG GTTTTTACAC GACGCAGCTT CTGCGTCGAA AAAGTTAGC
27301 27401 27501	27601	27801	28001	28201	28301	28601 28601 28701 28801

Figure ISR

28901	CHICATACAAC	CTTGAAGTCA	GGCT*CCTGG	ATCTCARCAT	CHUNCHTRAG	CHIGAGICA GRITICINY ANGIONICH CHGACTITICG CEAGEACCTG TECCGGAT THGITICCAGT CEAACTACAG CGACCAGTC	TCCCGCGGAT 1	PROFINCEAGT (	CCAACTACAG	כמאכככאכייכ
 	CCCCATGITIC	GAACTTCAGT	CUTANCERIC	TACAGTUGTA	CACTGAAACC	GAACTTCAGT CLUMICUARY TACACTICATA CACTGAMACE GGTEGITAME ARGGERECTA AACAAGGECA GGTEGATGTE GETHAGTUA	AGGCGCCTA 1	ANCAAGGTCA (	COLLONICAC	GCTNAGGTVARG
29001	TAACAGAGAT	GACCAMCACA	ACCAACGCG	כבפכבמניבעכ	CCIGACTTACA	GACCINGACIA ACCIMICACIA CEGECEGETAC EGGACITIACA TETACEACIA ATACACEECA AGTITICIGE TITUTICAATA ACTIXIGATAA	ATACACCCCA 1	AGITTICIDEC '	FFEGRENATA	ACTICIGATIVA
	ATTOTCTC7A		TOSTROCACC	CCCCCCCATC	CCCTVAATCT	ENGETTOTOT TOSTTOCICC COCRECIANTS CICCITIANICS AGAINSTICT TATGRINGOT TCAMDAGGG AACAGITAT TOACCEANT	TATCHYCOOT	TCAMGACGG 1	<b>AAACAGTITAT</b>	TORUCCTAIL
29101	CHROOCEARG	-	CCATAGCGCT	TATITION	TCK;CTTATTA	NEGRECITY CENTRICISCY TREFITTION TREACTION TRANSPORMS CANCECTES CANADACTOR ANCOCOCCO ACCONTE	CATCTCCTGC (	CTAMARCCCA !	AACOCOCCCO	ACCACCCATC
1	GAACCCGTAC	ACCACCAAGA	GCTATCGCGA	NTACAMENT	ACGGANTANT	ACCACCAADA GGTATCGCGA ATACAAACAT ACGAAATAAT AATACACAGA GTAGACGACG GATTFCGCGT 1TGCGCQGGC TCGFFFFFTAG	GTAGACGACG	GATTICGCGT '	macacadac	TOGT (CT.I'AG
29201	TATAGICCCA	TCATTGTGCT	ACACCCANAC	MATGATGA	TUCATAGATT	TCATTOTICT ACACCCAAAC AATGATGAA TICATAGAT GAACGGAATG AAACAGATG TCTTTTCTCT FACAGTATGA TTAAATGAAA	ANACACATOT '	activities .	TACAGTATGA	TTANATCACA
, !	ATATCAGGGT	_	TGTGGGTTTG	TTACTACCIT	AGGTATCTAA	AGNACACGA TETEGGETTE TIACTACCIT AGGIATCIAA CCTGCCTGAC TITCICIACA AGAAAAGAGA AIGICATACI AATTIACICI	TTTCTCTACA .	ACANANGAGA	ATGTCATACT	AATTTACTCT
	•	Xhot			•					
29301	CATCATTCCT	_	TATTACTGAC	CCTTGTTGCG	CTITITION	CONCENTRAL PARTACTORC CUTTATIBLE CITITITIES CONSCIENCE ATTORCTORC STRUCTOR TODARDADA CTOCATTO	ATTOGCTOCO	GIFFICTCACA	PCGAAGT'AGA	CTCCATTC A
		_	ATAATGACTG	CCANCANCOC	CHANNANCAC	GCTCANANT ATNATGACTG GGAACAACCG GAAAAAACAF GEACGAAGTTS TYACCGACGC CAAAGAGTGT AGCTTCATCT GACGTAAA.A	1'MCCGACGC	CAAAGAGTGT	AGCTTCATCT	GACCTANO.T.
					Pstl	- F				
29401	GCCTTCACAG		TTACGGATTT	GTCACCCTCA	CACTCATCTA	TETATITIECT TTACEGNITT STENECETEN CRETENIETR CAGESTEATE ACTUTIONER TESCETITAT CEAGRICATT SACTOSONIT	ACTIGITICA	TCCCCTTTAT	CCAGTGCATT	GACTOGOTICE
	CCCAAGTGTC		AATGCCTAM	CAGTGGGAGT	GCGAGTAGAC	agataaacga aatgectaaa cagtgggagt gcgagtagae gtextagtag tencaccagt aggggaaata ggtcacgtaa etgacccaa	TCACACCAGT	AGCGGAAATA	GCTCACCTRA	CTCACCCAMA
							EcoRI)	_}		
29501	granded	- TOCATATORC	AGACACCATC	CCCAGTACAG	GGACAGGACT	T TICATATICE AGACACCATE CCCAGTACAG GOACAGGACT ATAGCTGAGE TTCTTWGAAT TCTTTAATTA TGAAATTAC TGTGACTTT	PTCTTMGAAT	TCTTTAATTA	TOMATITAC	TGTGACTTT"
	CACACGCGAA	ACCTATAGAG	TCTCTCTAGTAG	GOSTCATISTIC	CCTGTCCTGA	<u>ACGIATAGAG TCTGTRGTAG GOGTCATGTC CCTGTCCTGA TATCGACTCG AAGAATCTTA AGAAATTAAT ACTTTAAATG ACACTGAAAA</u>	ANGNATCTTA	AGALATTAAT	ACTITITAAATO	ACACTONANI
29601	CTOCTOATTA		ATCTGCGTTT	TOTTCCCCGA	CCTCCAAGCC	PFIGCACCET ATCITUTATION TOTACCEGA CETCCAAGCE TEAAAGACAF ATATCAAGCA GAITCACTEG TATATGGAAF ATTCCAAGIT	ATATCATGCA	GATTCACTCG	TATATOGAAT	ATTCCAAGIT
1	GACGACTAAT		TAGACCCANA	ACANGGGGGT	GGAGGTTCGG	aarchooga tagacocaaa acaacooct geagethcog agtitetgta tatagtacgt etaagtage atatacetta taacetheaa	TATAGTACGT	CTAAGTGAGC	ATATACCIFIA	TAAGGTTCAA
							Pst			
29701	CCTACAATGA		CTTCCGAAG	CCTGGTTATA	TCECABTICATE	ANANAGGAT CTITCCGAAG CCFGGTTATA TCCAATCAFC TCTGTTATGG TGTTCTGCAG TACCATCTTA GCCCTAGCTA TATATCCC+A	TUTTICTICAG	TACCATCITA	GCCCTAGCTA	TATATCCC
	CGATGITACT		GAAAGGCTTC	CGACCAATAT	ACCITTAGTAG	ITITICOCTA GAAGOCTIC GONCCAATAT ACGITAGIAG AGACAATACC ACAAGACGIC ATGGIAGAAT COOGAICGAT ATAINAGAI	ACAAGACGTC	ATGGTAGAAT	COOGATCGAT	ATATACKGAT
29801	CYPTCACATT		CAATAGATGC	CATGAACCAC	CCANCITICC	OBCINGANCE CAATAGATEC CATGAACCAC CCAACTITICC CCGCGCCCCG TATGCTTCCA CTGCAACAAG TIGITGCCGG CGGCTTTTTT.	TATOCTTCCA	CTCCAACAAG	TTOTTGCCCG	COCCUTINENC
	CONCTOTAR		: GTTATCTACG	s GTACTTGGTG	GOTTICAAAGG	CCGACCTTGC GTTNTCTACG GTACTTGGTG GGTTGAAAGG GCCGCGGCG ATACGAAGGT GACGTTGTTC AACAACGGCC GCCGAAAAA 1	ATACGAAGGT	GACGITOTIC	MCMCGGCC G	GCCCAAAACA 1

CICTOCACCC ITATTANGAC CCHITGCGGT CTCAAAGATC TTATTCCCTT TAACTAATAA AAAAAATAA ITAAGCATCA CTTACTTAAA ATCAGTTAGC GAGACGTGGG AATAATTCTG GGACACGCCA GAGTTTCTAG AATAAGGGAA ATGATTATT TTTTTTATT ATTTCGTAGT GAATGAATTT TAGTCAATCG TRACOGRAINC TITAGERISAT GAAATTAGAT TOTCOTOCTO TACTGACTOT GGGATCTAGA TOTTTACCTO GIVENTICATO CACADADACCE CATTACEATA ACTERICACIE CYCTARAVAC COANGOCTOS ATTERICACIO CTTOTEAGG ACCTGARATA CAGACECEA CONTROL CAGACECEA CAGACEA CAGACEA CAGACECEA CAGACEA CAGACA CAGAC ACCTGAGGAT ACCITICACIC ACCICICACAG ANAICACITA CITITAATICTA ACAGAAGAA ATGACTGACA COCTAGAACT AGAAAATGAC ARACGENARIG CAOCGGEEGA GEAACAGEGE ATGAATEAAG AGETEEAAGA CATGOTTAAC TTGCACCAGT PETGEGINECE GINEREGICT COTTGINECE TACTTABITIC TEGROSTICT GTACEAATTG AACHIGHEA CCAAGCGT\*\* GCTTTCTTAK:T AAGITIGCCAA POCCOSTITICA GROCATICCTG TCATTATEST GOCCTGTGGC GGAATCGATG TTCAACGGTT ACHICLANAST CALCTACGAC AGTANTACCA CCGGACACCG CCTPNGCTAC TOGNAGACCC CCTGCTAGAA GCACGATCTT CICCIANAGE GAGCATTITCG TCGCAGCGGG GGAATTATTA CAGAGCAGCG GICTCGICCC TATCTTTGT ATAGAMACA CCAGCCANTC AGCCTCGCCC CCTTAATAAT GCANANGGGG GRANTTOCTO CITTANCCAC COTCGGTTMG CONTINCE 30301 30201 30101 29901 30001

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30401	AATHCTGT	CCAGTITATI	CAGCAGCACC	rectranect	CCTCCCAGGT	CTGGTATTGG	AGCTTCCTCC	TOGCTOCAAA	CTITICACAC	AATCTAAATG
	TTTAAAGACA	GGTCANATAN	Greeneeree	AGGAACGGGA	ההאימהדביה	GACCATAACG	Trganggagg	ACCOACOTIT	GAAAGAGGTG	TTACATTIAC
30501	GAATGTCAGT	-	TCCTGTCCAT	CCGCACCCAC	TATCTTCATG	THEFT	TGAAGCGCGC	AAGACCGTCT	GAAGATACCT	TCAACCCCCT
	CTTACACTCA	AAGGACGACA	AGGACAGGTA	GCCGTCCGTG	ATAGAAGTAC	ANCANCITET	ACTITICATION	TTCTGGCAGA	CITICITATION	ACTTGGGG A
30601	GTATCCATAT	GACACODAAA	CCGGTCCTCC	AACTGTGCCT	TTTCTTACTC	Crecentrat	ATCCCCAAT	OCCUPATIONS	AGAGICCCC	TGGGGT'AC'
	CATAGGTATA	CTOTOCCTTT	GCCCAGGAGG	TTGACACGGA	FIGACACGGA AAAGAATGAG	GAGGGAAACA	TAGGGGGTTA	CCCAAAGTTC	TCTCAGGGGG	ACCCCATGAG
				i.	. store					
30701	retricedec	TATCCGAACC	TCTAGTTACC	TCCAATGGCA	TCCAATGGCA TKICTTYSCOCT	CAAAATGGGC	AACAGACACT	CTCTGGACGA	GCCCGCCAAC	CTTACCTCC1:
	AGAMACGCGG	ATAGGCTTGG	AGATICANTIGG	AGGITACCGI	ACCOMPACTOR	GTTTTACCCG	TTGCCCGGAGA	GAGACCTGCT	ccooccomo	GAATGGAGE 3
30801	AAAATOTAAC			AANNANCCAA	GTCANACATA	MACCTEGRAM	TATETAGE	CCTCACAGIT	ACCTCAGAAG	CCCTAACTOF
	TITITACATIO	GTGACACTCG	COTOCACACT	TTTTTT	CAGTITICTAT	TICKACCTTT	ATAGACGTCS	GGAGTGTCAA	TOGAGICTIC	GGGATIGACA
30901	<b>GOCTOCCOCC</b>	GCACCTCTAA	TOGICOCOOD	CARCACACTC	ACCATGCAAT	CACARGECEE	GCTAACCGTG	CACGACTCCA	AACTTAGCAT	TUCCACCCAA
	CCGACGGCGG	CCTOGAGATT	ACCAGCOCCC	CITICICITAG	TESTACCITA	GTGTCCGGG	CCATTCCCAC	GIGCIGAGGT	TTGAATCOTA	ACCONTOCO P
31001	OGACCCCTCA			GCCCTGCAAA	CATCAGGCCC	CCTCACCACC	ACCGATAGCA	GTACCCTTAC	TATCACTOCC	TCACCCCC11"
	CCTGGGGGAGT	GICACAGICT	TCCTTTCGAT	COCCACCTT	GTACTCCCCC	CCACTOCTOG	TOGETATEGE	CATCCCAATG	ATACTGACGG	ACTICICICION
31101	TAACTACTGC				<b>OCCCATITIAT</b>		GAMMETAGG	ACTARAGTAC	OGGGCTCCTT	TOCATGTAL .
	ATTGATGACO	GIGACCATCG	ACCCCTAAC	TOANCIPTICE	COCOTANATA	TCTCTTTAC	CTTTTGATCC	TCATTTCATO	CCCCGAGGAA	ACCTACATTV
31201	AGACGACCTA				GICACTATTA		CTTGCAAACT	AAAGITACIG	GAGCCTTGGG	TITIGATICA
	Terroctional	THOTOLANCT	OCCATCGTTG	ACCAGGTCCA	CACTCATAAT	TATTATGAAG	GAACGITTIGA	TTTCAATGAC	CTCGGAACCC	NANCTAN .
31301	CAAGGCAATA	-		GGACTAAGGA	TRIATTETCA	MACAGACGC	CTTATACTEG	ATOTTACTTA	<b>TCCOTTTGAT</b>	<b>GCTCANAACC</b>
	GTTCCOTTAT	ACCITICAATT	ACATCGTCCT	CCTGATTCCT	AACTAAGAGT	THURCICO	GAATATGAAC	TACANTCAAT	AGGCNAACTA	CONOTITION
31401	AACTAAATCT	ANGACTAGGA	CAGGGCCCTC	TITITATAMA	CTCAGCCCAC		TTAACTACAA	CAAAGOCCITY	TACTTOTTTA	CAGCTTCAA
	TTGATTTAGA	TTCTOATCCT	GTCCCGGGAG	AAAAATATTT	averceeere	TTGAACCTAT	ANTTGATGTT	GTTTCCCGAA	ATGAACAAT	GTCGAAGT7' P
		Facilia			-					
31501	CAATTCCAAA			CACTGCCAAG	GOOTTOATGE	TTCACCCTAC	AGCCATAGCC	ATTAATGCAG	GAGATGGGCT	TOMATTEC: P
	GITAAGGTTT	TTCOAACTCC	AATTCGATTC	GYGACGGFYC	CCCANCTACA	AACTGCGATG	TCGCTATCGG	TAATTACGIC	CTCTACCCGA	ACTTANACCA
31601	TCACCTAATG			ANAACAMAA	TRACCATOO	CCTAGAATTT	GATTCAAACA	AGCCTATGGT		CONNCTERCE
	ACTOCATTAC	orocranoro	TITACCCGAG	THISHIT	AACCGGTACC	CGATCTTAAA	CTANCITICE	TCCGATACCA	ACCATITICAT	CCTTGACCCIG
31701	Tractitica	CACCACAGGT	OCCATTACAG	TAGGNAACAA	<b>ANATANTONT</b>	AAGCTAACTT	TUTOGRACIONE	ACCAGCTCCA	TCTCCTAACT	GTAGACTAAA
	AATCAAAACT	GICCHOICCA	CGGTAATGTC	Ancermen	TTTATTACTA	TICGATTGAA	ACACCTRAGIG	TGCTCGAGGT	AGAGGATTGA	CATCICATIT
31801	TOCAGAGAM	GATGCTAAAC	TCACTITIOGF	CTTAACAAA	TOTAXCACTO	MATACTTGC	TACAGITITICA	GTTTTGGCTG	TTAAAGGCAG	THIGHERCA
	ACOTOTOTA	CTACGATITIG	AGTGAAACCA	DAATTICTTIT	ACACCGTCAG	TTTATGAACG	ATCTCAAAGT	CAAAACCGAC	AATTTCCOTC	AAACCGAGGT
11901	ATATCHMAAA		-	ATTATAAGAT	THENCONANA		CTANACAATT	CCITICCTOGA		TR. JANCETETA
	TATAGACCTT	GTCAAGTTTC	ACGAGTAGAA	TAATATICTA	AACTCCTTTT	ACCTCACGAT	CATTICTTAA	GGAAGGACCT	OCCUCITATA	ACCTICAAAT
		- A - A - A - A - A - A - A - A - A - A								
32001	CITTACCICT	GAAATGGAGA TCTTACTGAA CTTTACCTCT AGAATGACTT	CCGTCTCGGA	ATACAMACGC	TCTTCCTATT	TACGGATTGG	TATCACCTTA	AGGTTTTNGA	GIGCCATTIT	GACGGTTTTC
:										

Figure 15T

		Action a contract	Popular Parameter							
10176	ATTGTAACAG	TCAGTTCAAA	TOANTTINGE	אניזריזריזריזריזריזר זריזריזריזריזריזר	TTTKX:ACAIT	GIGATICSTA	ATGTGATTTG	CCATCTGTCC	TTTOTCCTCT	CACAMCTC.A GTGTTTGAG:1"
32201	AGTOCATACT TCACGTATGA	CTATOTCATT	TTCATCGGAC AAGTACCCTG	WX#TC#GGCC ACCAGACGGG	ACAM TACAT TOTAMATITA	FANTGAMATA ATTAC:TTTAT	THECTACAT	CCTCTTACAC	TTTTTCATAC	ATTRECENY: TAACRICITY:
32301	AATAAAGAAT	CGIFFICTGIFF	ATGITHTCAAC	CACAMATANA	TTCANTITE'A AAGT'PAACGT	GAAAATTTEA	NTICATITITE TCAGTANAAA	CATTCAGTAG	TATAGCCCCA	CCACCACATA
32401	GCTTATACAG	ATCACCGTAC TAGTGGCATG	CITAATCAAA	CTCACACAAC	CCTACTATTC	AACCTACCAC	CHYCCTCCCA	ACACACAGAG TGTGTGTCTC	TACACAGTCC	TTTC: PCCC1 Y:
32501	GCTOGCCTTA	ANANGCATCA	TATCATGGGT	AACAGACATA	TTCTTAGGTG		CACAGITATICE	TGTCGAGGCA	AACGCTCATC	AGTONTATT . TCACTATA
32601	ATAMACICCC TATTICAGGG		ACTTAAGTTC TGAATTCAAG	ATCHCCTCT TACACCGACA	ההאירדים הדים מהדינים הנים ב	ACCACAGGG TOCTGTCCAA TCGJTGTCCG ACGACAGGTT	TCCTCTCCAA	CTTGCCGGTTG	CTTAACGGGC	GOCHANGAN:
							Pictor Policy Control of the Control			
10/26	**************************************	CTACATGGGG	GTAGAGTCAT CATCTCAGTA	MATCCACCAT	CACCATACAGE	CLASTAGNACT GCAGCAGLOC GCCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGGA CGTCGTGGTCGCG	CCAGCAGCUC CONTGINEGEO	COCITATITO	ACCIACCOCO	CCCCACCA
	Pati									-
32801	CCTGCAGGAA		CAGTOGICTC	CTCAGCGATG	ATTCGCACCG	CCCGCAGCAT	AAGGCGCCTT	GICCICCGGG	CACAGCAGCG	CACCCTOA!
	GGACGICCIT	ATOTTOTACC	GTCACCAGAG	GAGTCGCTAC	TAAGCGTVANC	GGGCGTCGTA	TTCCCCCCAA	CAGGAGGCCCC	Grercercec	GTGGGACT'''
. !			Pall							
32901	TCACTTAAAT		ACTOCAGCAC	AGCACCACAA	TATTGAT	ANTECEACAG	TGCAAGGCGC	TGTATCCAM	GCTCATGGCG	GOGACCACAG
	AGTGAATTTA	GICCIOICAT	TCACCTCCTG	recreenent	ATAACAAGTT	TTAGGGTGTC	ACCITICONCO	ACATAOGTTT	CGNOTACCGC	CCCTCOTCTC
33001	AACCCACOTG	GCCATCATAC	CACAAGCGCA	GCTAGATTAA	GTAGGGACCC	CTCATAAACA	CUCTUGACAT	ANACATTACC	TCTTTTGGCA	TCTTCTAATT
	TROOTIGCAC	COGTACTATG	Grerregeer	CCATCTAATT	CACCGCTGGG	GAGTATITIGE	GCGACCTGTA	TITGTAATGG	AGANAACCOT	ACAACATTAA
		Kpri								Pill
33101	CACCACCTCC	U		TAAACCTCTG ATTAAACATG GCGCCATCCA	GCGCCATCCA	CCACCATCCT	MACCAGCTO	GCCAAAACCT	3003303330	TATACACTTS.
	GTGGTGGAGG	OCCATOGTAT	ATTTOCAGAC	TAATTTICTAC	CGCGGTAGGT	GOTGOTACCA	TTTCCTCGAC	COCTATAGGA	دووودووددو	ATATETENE:
	E 3							Econy		
33201	ACCORACCOG	GACTOGAACA	ATGACACITY	AGAGCCCAGG	ACTECHTANCE	ATCGATENTE ATCCTEGTER	ATCCTCGTCA	TCATATCAAT	CTTCCCACAA	CACAGGCACA
	Tecerroace	CTCACCTTOT	TACTUTCACC	rereastree	TONOCATTOO	TACCTAGING	TACCAGCAGT	ACTATAGETEA	CAACCGTGTT	GTGTT:CGTGT
	•									Pstl
33301	COTOCATACA	CTRUCTICADO	ATTACAAGCT	CCTCCCGCGT	TAGAACCATA	TECCAGGGAA	CAACCCAFTC	CTGAATCAGC	<b>GTAAATCCCA</b>	CACTRCAGGG
	GCACGTATGT	DANGGAGTCC	TAATGTTCGA	GGAMGGCGCA	ATCTINGTAT	AGCGTCCCTT	CTTOGGTANG	GACTTAGTCG	CATTIAGGGT	GIRACCIICE:
33401	AAGACCTCCC	ACCTAACTCA	CGTTGTGCAT	TOTOMAGE	TTACATTCGG	CENTRACTOR	ATGATCCTCC	AGTATOGTAC	CCCCCCTTTC	TGTCTCANA
	TTCTOGAGCC	TOCATTOACT	CCAACACGTA	ACAGITITICAC	ANTOPAAGCC	ניהונימינים	TACTAGGAGG	TCATACCATC	GCGCCCMAG	ACAGAGTETE
33501	COACCTACAC	GATCCCTACT	GTACCGAAGTG	COCCONGACA	ACCCACACATCG	Triplication	AGTGTCATGC	CANAITGGAAC	OCCOGACUTA	CICATATTY.
	CCTCCATCTO	CTAGGGATGA	CATUCCICAC	CCGCTCTGT	TOCCTCTAGG	ACAACCAACA	TCACAGTACG	GTTTACCTTG	CCCCCTCCAT	CAGTATAAN

n Gil

33601	CTCAACCAAA	ACCAGGTGCG TORTICCACGC	GGCGTGACAA	ACAGATOTOC TOTOTAGAGG	CAGAGGGAG	MYGCGCTTA GATCGCTCTG TGTAGTAGTT GTAGTATATC CACTCTCTA AGGGGGAAT CTAGGAAGA ACATCATCAA CATCATATAG GTGAGAGAGT!	CTANCOCACAG	TCTAGTAGTT ACATCATCAA	GTACTATATC CACTCTCTTA CATCATATAG GTGAGAGACT	CACTCTCTTA GTGAGAGATI'
33701	AAGCATCCAG	OCOCCCCTO	GCTTCGGGTT				TGATAACATC		GANTAAGCCA	CACCCAGCC
!	Tregradore	CGCGGGGGGCAC	CGAAGCCCAA	GATACATITIG	AGGAAGTACG	COULCACOGG	ACTATTOTAG	GTCGTGGCGT	CTTATTCGGT	GTGGGTCGCII
33801	ACCTACACAT	restrended	AGTCACACAC	GGGAGGAGCG			CHALLILLIA	Trafficcada	AGATTATCCA	AAACCTT WAA
	TCGATCTCTA	AGCAAGACGC	reacterers	CCCTCCTCCC	CCTTCTCGAC	CHICTIGGTA	CHARAMARA	ANTAAGGTTT	TCTAATAGGT	TTTCCACTT
	Bott	•								
33901	ATGAAGATCT	ATGABOATCT ATTAAGTGAA	CGCGCTCCCC	Teegetages	TYGICANCT	CTACAGCCAA	NGNACAGNTA	ATCCCAPTIO	TAAGATGTTO	CACANTOGCT
	TACTTCTAGA	TAATTICACTT	BCCCGAGGGG	AGGCCACCC	ACCAGTITICA	GATCTCGGTT	TCTTGTCTAT	TACCGTAAAC	ATTCTACAAC	GTGTTACCCIA
34001	TCCAAAAGGC	AAACGGCCCT	CACCTCCAAG	TEGACGTAAA	GGCTANACCC	TTCAGGGTGA	ATCTCCTCTA	TAMACAPTICC	ACCACCTTICA	ACCATGCCCA
	AGGITTICCO		<b>OTGCAGGTTC</b>	ACCTGCATT	CCGATTTCGG	AGTCCCACT	TAGACCACAT	ATTTOTANGO	TCGTGGNAGT	rectaceast
14101	AATAATTCTC	ATCTCOCCAC	CTTCTCANTA	TATCTCTAAG	CANATOCOGA	ATATTAAGTC	CGGCCATTGT	AAAAATCTGC	TCCACACICCC	CCTCCACCTT
	TTATTAAGAG	_	_	ATAGAGATTC	GTTTAGGGCT	TATAATTCAG	CCCCCTAACA	TITITAGACO	AGGICTCOCO	GGAGGTGGAA
34201	CAGCCTCAAG	CAGCGAATCA	TGATTGCAAA	AATTICAGGTT	CCTCACAGAC	CTUTATANGA	TTCANAGCG	GAACATTAAC	AAAAATACCO	CGATCCCGTA
	GECOGRAFIC		_	TTAAGTCCAA	GGAGTGTCTG	GACATATTET	AAGTTTTCOC	CITICIAATIG	TITITATOOC	GCTNGCGCA1
34301	GOTCCCTICG	CAGGGCCAGC	TGAACATAAT	CGTGCAGGTC	TECACOGACE	AGCGCGCCA	CTTCCCCGCC	AGGAACCATG	ACAAAAGNAC	CCACACTGAT
}	CCAGGGAAGC	Greceogred	ACTITATITA	GCACGITCAG	ACGIGCCTOG TCGCGCCGGT	TCCCCCCGGT	DANGGGGGGG	recreasing	rerrrrrr	GETGTGACTA
					E T	102				
34401	TATGACACGC		ATACTCOGAG CTATGCTAAC	CAGCGTAGCC	CCGATGTAAG CTTGTTGCAT	CITIGITISCAT	OGGCOGCGAT	ATAAAATGCA	AGGTOCTOCT	CANNANATO
1	ATACTOTOCO		GATACGATTG	GTCGCATCGG	<b>GOCTACATTC</b>	GAACAACGTA	CCCCCCCTA	TATTITIACGE	TCCACGACGA	GTTTTTTAG.
34501	OCCAMBEE	COCCCANAAA	AGAAAGCACA	TCGTAGTCAT	CCTCATGCAG	NTANGGCAG	GTAAGCTCCG	GNACCACCAC	AGAMANAGAC	ACCAPPIPITIC
! !	CCGTTTCOGA	OCOCOTITITI	remicolor	AGCATCAGTA	CGAGTACGTC	TATTTCCGIC	CATTCGAGGC	CITIGOTOOTO	TCTTTTCTO	TGGTANAANG
34601	TCTCAAACAT	prerocodor	TTCTGCATAA	ACACAMMTA	ANATHACANA	MANCATITIN	AACATTAGAA	OCCIONETTA	CAACAGGAAA	AACAACCC1"!
	AGAGITITOTA		AAGACGTATT	TCTGTTTTAT	TFFATTGFFF	TTTTGTAAT	TICHMATCH	COGNCAGAAT	OFICICATIF	TTGTTCCCAA
34701	ATAMOCATAA	GACCICACTAC	_	OCCITICACCUT	AAAAAACTG	GTCACCOTGA	TTAMANGCA	CCACCCACAG	CICCICONIC	ATGICCEGAG
	TATTCGTATT	CTGCCTGATO	CCGGTACGCC	CCCACTCCCA	TITITITIENC	CASTGGCACT	AATTTTTCGT	<b>GGTGGCTGTC</b>	GAGGAGCCAG	TWINGREETE
34801	TCATAATOTA	AGACTCOOTA	AACACATCAG	GITGATICAC	ATCOSTCAGE	GCTAANNGC	GACCGANATA	GCCCCCCCCCCA	ATACATACCC	GCACACACTOTAG
	AGTATTACAT	TCTGAGCCAT	TIGIGIANT	CAACTAMETG	TAGCCAGTCA	CGATTITICO	CTOGCTTTAT	COOCCCCCT		CGICCGCATC
34901	AGACAACATT	ACAGCCCCCA	TAGGAGGTAT	AACAAMITTA	ATAGGAGAGA	ANANCACATA	AACACCTGAA	AVACCETECT	_	AATAGCACCC
	TCTGFFOTA	-	ATCCTCCATA	TICHTERANT	TATCCICTOR	THTICHCIAL	TTCTCCACTT	TTTGGGAGGA		TTATCGTOOG
35001	TCCCGCTCCA	GAACAACATA	CAGCGCTTCC	ACAGCGGCAG	CCATAACAGT	CAGCCTTACC	AGTANAAAG			CACTCGACA:
	ACCOCCACCT	· CTIGHTGFAF	CTCGCGANGG	TOTOGOCOTO	GGTATTGTCA	GICCGAATGG	TCATTITITE	TTTTKEGATAA		GIGAGCTGTB
35101	COCACCAGET	· CAATCAGTCA	CAGTGTANA	AAGGGCCAAG	TGCAGAGGGA	<b>GTATATATAG</b>	GACTAAAAAA	TGACGTAACG	_	ACANAMACA
	CCGTOGTCGA	CITTAGTCAGT	GTCACATTTT	Trecedente	ACGICITORICA	CATATATATC		-		KILLINGE
35201	CCCAGAAAAC	: COCACOCOAN	CCTACGCCCA	GNAACGNAAG					TCCCACGTTA	CONCACTICC
•	GGCTCTTTG	GOSTICTITIVE GEORGEGETT	GGATGCGGGT	CTTTGCTTTC	CCTTTTTGG	GTGTTGAAGG	AGTITIAGCAG	TCAACCCAAA	AGGGTGCAAT	GCAGTUAAGG

figure 15V

# pMRKAdSgag MER682

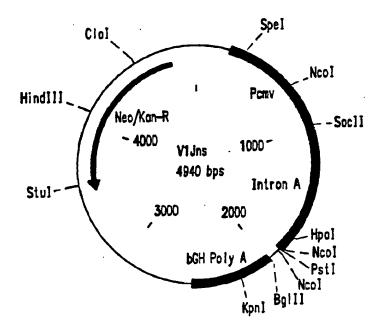
35401 CACCCCTCA 95501 CCCATTATOA 95701 GCCAGCAAA COCTCOTTTT 35701 GACAGCGCT 35801 CTCCACCGCT 35801 CTCCACCGCT 6ACAGCGGA 35901 TGCACCACC ACCTCTTGA	Tribute Stranger				•				
	_				THT.	Foofil		•	
	_	GCCTTCAATC	CANANTANGG	TATATTATTG	TACTACATT ,	TTAMBAATTC	GGATCTGCGA CCTAGACGCT	COCCAGOCTO	CTACCCOANG
	JA THEMETOGE	Treceses	ATCGGGATGC	CCGCGTTGCA		TCCAGGCAGG	TAGATCIACGA	CCATCAGGGA	CAGCTTCAAG
	T AAGAAGAGCG	AAGGCCGCCG	TAGCCCTACG	CCCCCAACGE	CCCCTACCAC	AGGTCCGTCC	ATCTACTGCT	GGTAGTCCCT	GICGAAGTTC
	NA GOCCAGGNC	CUTAAAAAGG	CCCCOTTGCT	GGCGTTTTTC	CATAGGCTGC	GCCCCCCTGA	CGAGCATCAC	AAAAATCGAC	OCTCAAGTCA.
		GACTATAAAG		TTTCCCCCTC		CONOCOUNCE	CCTOTTCCGA	CCCTGCCGCT	TACCGGATAC
	CT TYGGGCTGTC	CIGATATITIC	TATOGECOC	AAAGGGGAC	CTTCGAGGGA	CCACCCCAGA	GCACAAGGCT	CCCACGCCGA	ATGGCCTATY:
	CT PTCTCCCTTC	CCCTTCGCAC	GCGCTTTCTC	ATAGCTCACG TATCGAGTGC	CTGTAGGTAT	CHCANTINCOS	TCTAGGTCOT ACATCCAGCA	TCCCTCCAAG ACCCAGGTTC	CTCOCICTOD.  GACCCGACA!
	EC CCCCGFFCAG	CCCGACCOCT	GCGCCTTATC	CGGTAACTAT		CCAACCCGGF	AAGACACGAC	TTATCGCCAC	TOOCAGCAGY
	GO GOOGCAAGTC	GOGCTCGCCA	CGCGGAATAG	GCCAFFGATA	GCAGAACTCA	GGTTGGGCCA	Treferene	AATAGCCCTC	ACCENCENC
	AC AGGATTAGCA	GAGCGAGGTA	TOTAGGCGGT ACATCGGCCA	CCATCACAGAGT	ACAACTTCAC	GTOGCCTAAC	TACCOCTACA	CTAGAAGGAC	AGTATTTGGF TCATAAACCA
				THESTAGETE	TTGATCCGGC	ANACANACCA	CCCCTGGTAG	COOTCOTTTT	THEFTINE
TAGACGCGAG	AG ACGACITICOS	TCANTGGAAG	centracte	NACCATCGAG	AACTAGGCCG	THEFT	GGCGACCATC	GCCACCALAA	AAACAAACCT
36201 AGCAGCAGAT TCGTCGTCTA	AT TACGCGCAGA	AAAAAAGGAT	CTCAAGAAGA	TCCT**********************************	MAMGATGC	CCAGACTISCO	TCAGTOGNAC AGTCACCTTG	CITITICACTO	GTTANGGGA" CAATTCCCTA
36301 Trrootcate	TO AGATTATCAA	AAAGGATCTT	CACCTAGATC	CTTTTAAATC	ANTCTAANST	ATATATGAGT	AAACTITGGIC	TOACAGITAC	CAATACTEA
36401 TCAGTGAGG			TATTICGITIC	ATCCATAGTT		CCGTCGTGTA	GATAACTACG	ATACGGGAGG	GCTTACCATY:
36501 TOCCCCAOT				CCGCCTCCAG	ATTTATICAGE	AATAAACCAG	CCAGCCGGAA	GGGCCGAGCG	CAGAAGTGGT
ACCGGGG			GOCTCCCACT	OCCCGAGGTC	TAMATAGICO	TATTIGGIC	GOTCOOCCIT	CCCOCCICCC	GICTICACCA
36601 CCTGCAACTT GGACGTTGAA	TT TATCCOCCTC AA ATAGGCGGAG	CATCCAGTCT GTAGGTCAGA	ATTAATTGTT TAATTAACAA	CGCCCTTCG	TAGAGTAAGT	AGTTCGCCAG TCAAGCGGTC	TTAATAGTITE AATTATCAAA	CCCOTTCCAA	CANCOGTANC
36701 CTACAGGCAT GATGTCCGTA	AT COTOCHOTCA	CCCTCCTCGT	TTOGTATGGC AACCATACCG	TTCATTCAGC	TCCGGTTCCC AGGCCAAGGG	AACGATCAAG TTGCTAGTTC	GCGAGTTACA	TGATCCCCCA ACTAGGGGGT	TOTTGTCCAA
		Peti	_}						
36801 AAAAGCGGTT	AN TITISAGGAAGE	GTCCTCCGAT	CONTROTONGA	ACTANGITING TCATTCAACC	CCCCACTICTT	ATCACTCATG	CANTACCONC	CACTGCATAA	TICTCTTACT
36901 GYCATGCCAT CAGTACGGTA				ACTUMCCAA TGAGTTGGTT	GAGTACTGA	GAATAGTGTA		GAGITIGCTCT	TACCCRACOF

figure 15W

# PMRKAd5gag MER682

CAACACCODA TAATACCOCO CCACATAGEA GAACTITAAA AGTXCTEATE ATTXAAAAAC GTACTACOGO GCGAAAACAC TCAAGGATCT TACGGGTGTTT GTTGTGCCCCT ATTATAGGCGC GCTGTATEGT CTIXAAATTT TVACGAGTAG TAACCTTTTG CAAGAAGGCC CGCTTTTGAG AGTYCCTAGA ATGGGAAAA	BABATCCAGT TCBATGTARC CCACTCGRIC ACTCAACTGA TCTTGARATA CTTTTACTT CACCAGGGT TCTGGGTGAG CAAAAGAG AAGGCAAAAT CTCTAGGTCA AGCTACATTG GOTGAGCACG TCGGTTGACT AGAAGTGTTA GAAAATGAAA GTGTTCGCAA AGACCCACTC GTTTTTGTCC TTCGCTTTTA	TAT TOTOTOATKIA ATA ACAGAGTAKII	HAA CCATTATTA'	5
TCAAGGA	CAAAAAC	TCACAGGT AGTCCCA	GTCTAAG	ID NO: 2:
MAACTC	CCCACTC	GCATTTA	ACCTGAC	F (SEQ
8 8	5 5	\$ E	5 8	ATTA
GANGANGCCC	CACCACCETT	Caatai fatt Gttataataa	CCCGAAAAGT	Bantell  WANTER  CA TUCGAATTCT  CT AGGCTTAAGA
ATTCACAAAAC TAACCTTTTTG	CTITTACTTT GAAAATGAAA	CTTCCTTTT	CCCTCTATTTC GCCTCTAAAG	Bar www. AAGAATTGGA TYCTTAACCT
AGTRETEATE TYTAGGAGTAG	AGAACTCTA	TACTCATACT ATTACTATEA	AGGGRETHTCG TCCCCAAGGC	TTICGICITIC
GAACTTTAAA	ACTEANETEA TOCGETICACE	AAATGTTGAA TTTACAACTT	ATAAACAAAT TATTTGFFTA	CACGARACCC
CCACATAGEA	CCACTCGTGC	GGCGACACGG CCGCTGTGCC	ATTTAGAAAA TAAATCTTTT	ATAGGCGTAT TATCCGCATA
TANTACCOCO ATTATOGOCO	<b>TCGATOTAAC</b> AGCTACATTO	AGGGAATAAG	ATTTCAATGT TAAACTTACA	Bamel WILLIAM STATEMENT ACCTATANA ATARGEGIAT CACGAGGEC TTICGTCTIC ANGANTICA TCGAATTET TAAT (SEQ ID NO: 27) GTACTOTAAT TGGATTIT TATECOCATA GTGCTCCGG AAAGAGAAG TTCTTANCCT AGGETAAAA ATTA (SEQ ID NO: 28)
CAACACGOOA	GAGATCCAGT	CCGCGTFFFF	GCCGATACAT	CATGACATTA GTACTGTAAT
			37301	37401

Figure 15X



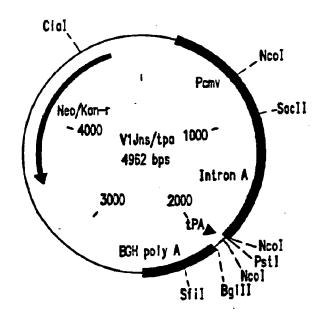


FIGURE 16

GCAGTGGCCCCTGACTGAGGAGAAGATCAAGGCCCCTGCTGGAAATCTGCACTGAGATGGAGAAGGAGGGCCAAAATCTCCA
sGInTrpProLeuThrGluCluLysIleLysAloLeuVolGluIleCysThrGluMeLGluLysGluGlyLysIleSerL
30 40 50

AGATTGCCCCGAGAACCCCTACAACACCCCTGTGTTTGCCATCAAGAAGAAGAACGACTCCACCAAGTGCAGGAACCTGGTG
yslieGiyProGiuAsnProTyrAsnThrProVoiPheAiolieLysLysLysAspSerThrLysTrpArgLysLeuVoi
60 70

GACTTCAGGGAGCTGAACAAGAGGACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCCACCCGGCTGGCCTGAAGAA AspPheArgGTuLeuAsnLysArgThrGinAspPheTrpGTuVoTGinLeuGTyTteProHisProAloGTyLeuLysLy 80 90 100

GAAGAACTCTGTGACTGTGCTGCCTGTGCGGGATGCCTACTTCTCTGTGCCCCTGGATGAGGACTTCAGGAAGTACACTG slyslysSerVolThrVolLeuAloVolGlyAspAloTyrPheSerVolProLeuAspGluAspPheArgLysTyrThrA 110 120 130

CCTTCACCATCCCCTCCATCAACAATGAGACCCCTGGCATCAGGTACCAGTACAATGTGCTGCCCCAGGGCTGGAAGGGC IoPheTnrlleProSerlleAsnAsnGluThrProGlylleArgTyrGlnTyrAsnVoiLeuProGlnGlyTrpLysGly 140 150

TCCCCTGCCATCTTCCAGTCCTCCATGACCAAGATCCTGGAGCCCTTCAGGAAGCAGAACCCTGACATTGTGATCTACCA SerProAioliePheGinSerSerMetThrLyslieLeuGiuProPheArgLysGinAsnProAsplieVollieTyrGi 160 170 180

GTACATGGCTGCCCTGTATGTGGGCTCTGACCTGGAGATTGGGCAGCACAGACCAAGATTGAGGAGCTGAGGCAGCACCC
nTyrMetAloAloLeuTyrVolGlySerAspLeuGluIleGlyGInHisArgThrLysIleGluGluLeuArgGInHisL
190 200 210

TGCTGAGGTGGGGCCTGACCACCCTGACAAGAAGCACCAGAAGGAGCCCCCCCTTCCTGTGGATGGGCTATGAGCTGCAC euLeuArgTrpGlyLeuThrThrProAspLysLysHisGlnLysGluProProPheLeuTrpMetGlyTyrGluLeuHis 220 230

CCCGACACTGGGCTGCCCCCATTGTGCTGCCTGAGAAGGACTCCTGGACTGTGAATGACATCCAGAAGCTGGGGGC ProAspLysTrpThrVoIG1nProIIeVoILeuProGluLysAspSerTrpThrVoIAsnAspIIeGInLysLeuVoIG1 240 250 260

CAAGCTGAACTGGGCCTCCCAAATCTACCCTGGCATCAAGGTGAGGCAGCTGTGCAAGCTGCTGAGGGGCACCAAGGCCCC
yLysLeuAsnTrpAloSerGinlleTyrProGlylleLysVolArgGinLeuCysLysLeuLeuArgGlyThrLysAloL
270 280 290

#### FIGURE 17A

TGACTGAGGTGATCCCCCTGACTGAGGAGGCTGAGCTGGAGCTGGAGCAGACAGGGAGATCCTGAAGGAGCCTGTGCAT EuthrGluVollleProLeuthrGluGluAloGluLeuGluLeuGluLeuAloGluAsnArgGluIleLeuLysGluProVolHis 300 310

GCGGTGTACTATGACCCCTCCAAGGACCTGATTGCTGAGATCCAGAAGCAGGGCCCAGGGCCCAGTGGACCTACCAAATCTA GlyVoiTyrTyrAspProSerLysAspLeulleAloGiulleGinLysGinGlyGinGlyGinTrpThrTyrGinlleTy 320 330 340

CCAGGAGCCCTTCAAGAACCTGAAGACTGGCAAGTATGCCAGGATGAGGGGGGGCCCCACACCAATGATGTGAAGCAGCTGA rGInGIuProPheLysAsnLeuLysThrGIyLysTyrAIaArgMeLArgGIyAIaHisThrAsnAspVoILysGInLeuT 350 360 370

CTCACGCTGTGCAGAAGATCACCACTGAGTCCATTGTGATCTGGGGCAAGACCCCCAAGTTCAAGCTGCCCATCCAGAAG hrGluAloVolGinLyslleThrThrGluSerlleVollleTrpGlyLysThrProLysPheLysLeuProlleGinLys 380 390

GGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATTGTGGGGGGCTGAGACCTTCTATGTGGCTGGGGCTGCCAACAGGG uVollysleuTrpTyrGinLeuGtuLysGtuProlleVotGlyAloGtuThrPheTyrVolAloGtyAloAsnArgG 430 440 450

AAGACTGCCCTCCAGGCCATCTACCTGGCCCTCCAGGACTCTGGCCTGGAGGTGAACATTGTGACTGCCTCCCAGTATGC
LysThrAioleuGinAloileTyrLeuAloleuGinAspSerGiyLeuGiuVolAsnIieVolThrAioSerGinTyrAi
480
490
500

CCTGGGCATCATCCAGGCCCAGCCTGATCAGTCTGAGTCTGAGCTGGTGAACCAGATCATTGAGCAGCTGATCAAGAAGG ©LeuGiyIielieGinAioGinProAspGinSerGiuSerGiuLeuVolAsnGinIielieGiuGinLeulieLysLysG 510 520 530

AGAACGTGTACCTGGCCTGGCCCACAAGGGCATTGGGGGCAATGAGCAGGTGGACAAGCTGGTGTCTGCTGGC
!uLysvoiTyrleuA!oTrpvoiProA!oHisLysG!y!!eG!yG!yAsnG!uGinVo!AspLysLeuVo!SerA!oG!y
540
550

ATCAGGAAGGTGCTGTTCCTGGATGGCATTGACAAGGCCCAGGATGAGCATGAGAAGTACCACTCCAACTGGAGGGCTAT

1 leAr gl ys Voil eu Pheleu AspG i y i leAsplys Ai oG i naspG i u His G i u Lys Tyr His Ser AsnTrp Ar gA i oMe

560 570 580

### FIGURE 17B

GGCCTCTGACTTCAACCTGCCCCCTGTGGTGGCTAAGGAGATTGTCCCCTCCTGTGACAAGTGCCAGCTGAAGCGGGAGG tAloSerAspPheAsnLeuProProVolVolAloLysGiulieVolAloSerCysAspLysCysGinLeuLysGlyGluA 590 600 610

GCTGTGCATGTGGCCTCCGGCTACATTGACGCTGACGTGATCCCTGCTGAGACAGGCCAGGAGACTGCCTACTTCCTGCT AlovolHisVolAloSerGlyTyrIleGluAloGluVolIleProAloGluThrGlyGlnGluThrAloTyrPheLeuLe 640 650 660

GAAGCTGGCTGGCAGGTGGCCTGTGAAGACCATCCACACTGCCAATGGCTCCAACTTCACTGGGGCCACAGTGAGGGCTG

uLysLeuAloGlyArgTrpProVolLysThrlieHisThrAloAsnGlySerAsnPheThrGlyAloThrVolArgAloA

680
690

CCTGCTGGTGGGCTGGCATCAAGCAGGAGTTTGGCATCCCCTACAACCCCCAGTCCCAGGGGGTGGTGGCCTCCATGAAC IoCysTrpTrpAloGlylleLysGInGluPheGlylleProTyrAsnProGInSerGinGlyVoiVoiAloSerMelAsn 700 710

AAGGAGCTGAAGAAGATCATTGCGCAGGTGAGGGACCAGGCTGAGCACCTGAAGACAGCTGTGCAGATGGCTGTTCAT LysGluLeuLysLyslielleGlyGlnVolArgAspGlnAloGluHisLeuLysThrAloVolGlnMetAloVolPhell 720 730 740

CCACAACTICAAGAGGAAGGGGGGCATCGGGGGGCTACTCCGCTGGGGAGAGGATTGTGGACATCATTGCCACAGACATCC
eHisAsnPheLysArgLysGlyGlyIleGlyGlyTyrSerAlaGlyGluArglleVolAspIleIleAloThrAspIleG
750
770

AGACCAAGGAGCTCCAGAAGCAGATCACCAAGATCCAGAACTTCAGGGTGTACTACAGGGACTCCAGGAACCCCCTGTGG
InThrLysGTuLeuGInLysGInlieThrLys1ieGInAsnPheArgVoITyrTyrArgAspSerArgAsnProLeuTrp
780 790

AAGGCCCCTGCCAAGCTGCTGTGGAAGGCGGAGGGGGCTGTGGTGATCCAGGACAACTCTGACATCAAGCTGGTGCCCAG LysGtyProAtoLysLeuLeuTrpLysGtyGtuGtyAtoVotVotIteGtnAspAsnSerAsplieLysVotVotProAr 800 810 820

AAAGCCCGGCCAGATC; (SEQ ID NO: 3) Xx Boll (SEQ ID NO: 4)

FIGURE 17C

(within SEO 10 NO: 7) (within SEO 10 NO: 8) RoSerGiulleSerAigProlleSerProlleGluThrVolProVoiLysLeuLysProGlyMetAspGly 20 20 

FIGURE 18

WT		-42
OPT	- ÁTG GGC GGC ÁÁG TGG TCC ÁAG AGG TCC GTG CCC GGC TGG TCC	-14
WT	11 11 11 11 11 11 11 11 11 11 11 11 11	-84
OPT	- ACC GTG AGG GAG AGG ATG AGG GCC GAG CCC GCC GCC GAC	-28
WT	111 111 11	-126
OPT	- ÁGG GTG ÁGG AGG ÁCC GÁG CCC GCC GTG GGC GTG GGC GCC R V R R T E P A A V G V G A	-42
WT	- GTA TCT CGA GAC CTG GAA AAA CAT GGA GCA ATC ACA AGT AGC	-168
OPT	- GTG TCC AGG GÁC CTG GÁG ÁÁG CÁC GĆC ÁTC ÁCC TCC TCC V S R D L E K H G A 1 T S S	-56
WT	- AAT ACA GCA GCT ACC AAT GCT GAT TGT GCC TGG CTA GAA GCA	-210
OPT	- AAC ÁCC GCC GCC ÁCC ÁAC GCC GÁC TGC GCC TGG CTG GÁG GCC N T A A T N A D C A W L E A	-70
WT	- CAA GAG GAT GAG GAA GTG GGT TTT CCA GTC AGA CCT CAG GTA	-252
OPT	. CÁG GÁG GÁC GÁG GÁG GTG GGC TTC CCC GTG ÁGG CCC CÁG GTG Q E D E E V G F P V R P Q V	-84
WT .	- CCT TTA AGA CCA ATG ACT TAC AAG GGA GCT GTA GAT CTT AGC	-294
OPT	- CCC CTG AGG CCC ATG ACC TAC AAG GGC GCC GTG GAC CTG TCC P L R P M T Y K G A V D L S	-98
WT	- CAC TIT TTA AAA GAA AAG GGG GGA CTG GAA GGG CTA ATT CAC	-336
OPT	- CÁC TÍC CTG ÁAG GÁG ÁAG GGC GGC CTG GÁG GGC CTG ÁTC CÁC H F L K E K G G L E G L I H	-112
WT	- TCA CAG AAA AGA CAA GAT ATC CTT GAT CTG TGG GTC TAC CAC	-378
OPT	- TCC CAG AAG AGG CAG GAC ATC CTG GAC CTG TGG GTG TAC CAC S Q K R Q D I L D L W Y H	-126
WT	- ACA CAA GGC TAC TTC CCT GAT TGG CAG AAC TAC ACA CCA GGG	-420
OPT	- ACC CAG GGC TAC TTC CCC GAC TGG CAG AAC TAC ACC CCC GGC T Q G Y F P D W Q N Y T P G	-140

FIGURE 19A

WT		CCA	11	111	П	$\Pi$	11	11	111	H	$\Pi$	111	$\Pi\Pi$	111	111	-462
OPT	•	ĊĊC P	GGC		AGG R	F	P	L	T	F	G	W	C	F	K	-154
WT		CTA	11	11	11	111	11	11	111	11	11	111	111	11	11	-504
OPT	•	ĊŤG	ĠŤG V	CCC	GTG	GAG	CCC	GAG	aag	GTG	GAG E	GAG E	GCC A	AAC N	GAG E	-168
₩T		GGA	111	$\Pi\Pi$	111	111	- 11	1	111	11		- 1			11	-546
OPT	•	GGC	ĠĀĠ E	AAC	AAC	TGC	CTG	CTG	CÁC	CCC P	ATG M	TCC S	CAG	CAC H	GGC G	-182
WT		ATA	111	111	11	111	111	11	111	11	$\Pi\Pi$	111	$\parallel \parallel \parallel$	11		-588
OPT	•	ÁŤC	GAG E	GAC	ĊĊC	GAG	AAG	GAG	GTG	CTG	GAG	TGG	AGG	TTC F	GAC D	-196
WT	•	AGC	AAG										CTG	CAT	CCG	-630
OPT	-	TCC	AAG	CTG	GCC	TTC	CAC	CAC	GTG	GCC	AGG	GAG	CTG	CAC H	ĊĊC P	-210
WT	•	GAG								EQ I	D NO	:30)				-651
OPT	•	GAG	TAC	TAC	AAG	GAC	TGC	TAA	(c	onta EQ 1	ined D NO:	wit :10)	hin	SEQ	ID NO: 9)	-216

FIGURE 19B

VIJns/nef

VIUNISTIE! PSEI CATGGGTCTTTT<u>CIGCAG</u>TCACCGTCCTTGAG<u>AICT</u>GCCACC ATG GGC GGC AAG TGG TCC AAG TCC GTG CCC . M G G K W S K R S V P

SrfI B9111

CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGGAGAICIGCTGTGCCTTCTAGTTGCCAGC (SEQ 1D NO: 38)

H P E Y Y K D C \* (contained within SEQ 1D NO: 10:

V1Jns/nef(G2A.LLAA)

Psti Catrbastictiticigicalgricaccotictitagia<u>ilci</u>taccacc atg gcc ggc ang tgg tgc gtg ccc M A G K W S K R S V P

SrfI BallI . . . . CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGGALCTGCTGCCTTCTAATTGCCAGC (SEQ 1D NO: 39) H P E Y Y K D C \* (contained within SEQ 1D NO:14)

/lJns/tpanef & VlJns/tpanef(LLAA)

Psti Catibasictiticiocalgicaccostatatatictagatcacc atg gat gca atg ang aga ggg ctc tgc tgt gtg M D A M K R G L C C V

. . . . CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGGAGATCTGCTGTGCTTGTAGTTGCCAGC (SEQ ID NO: 40)

H P E Y Y K D C \* (contained withon seq id no: 16.)

### FIGURE 20

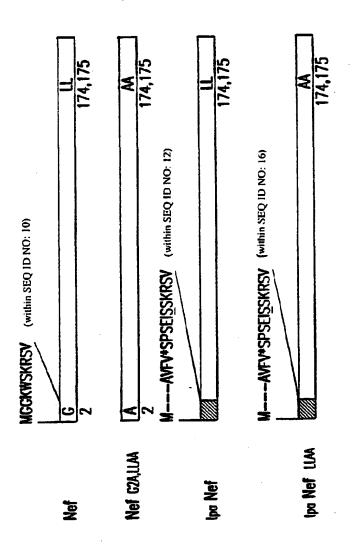


FIGURE 21

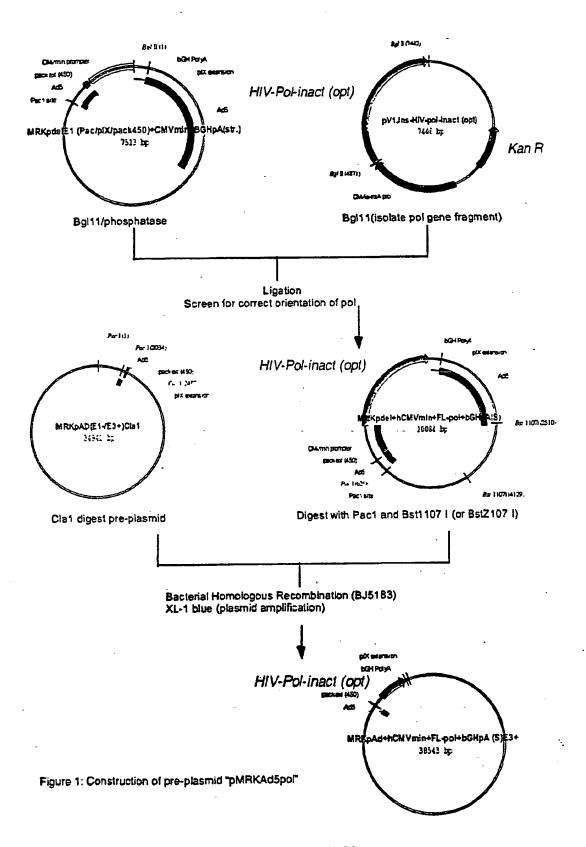


FIGURE 22

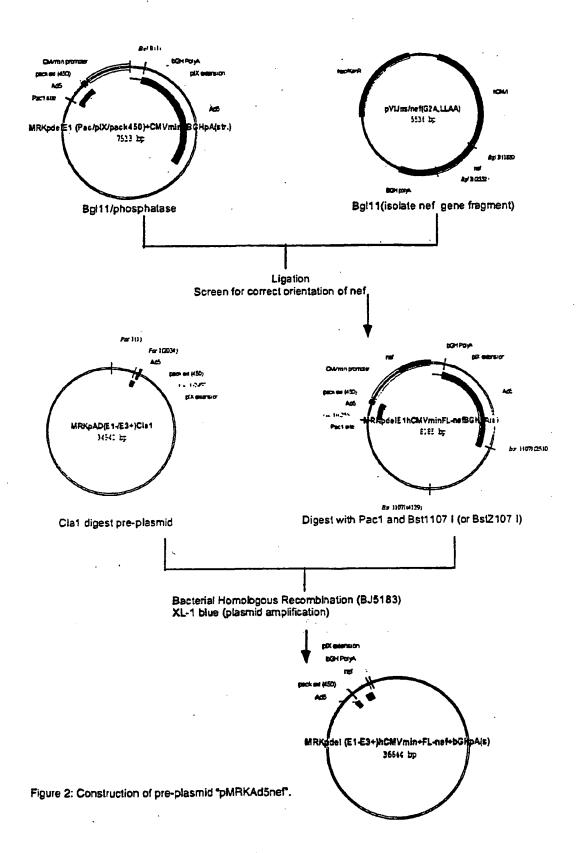
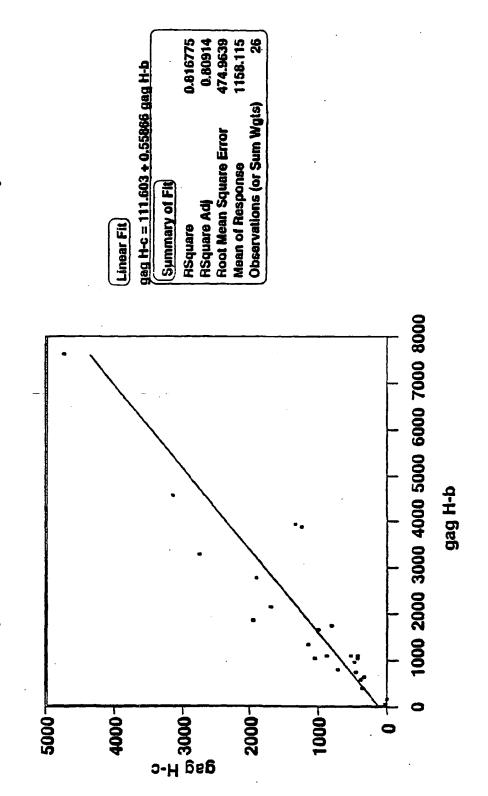
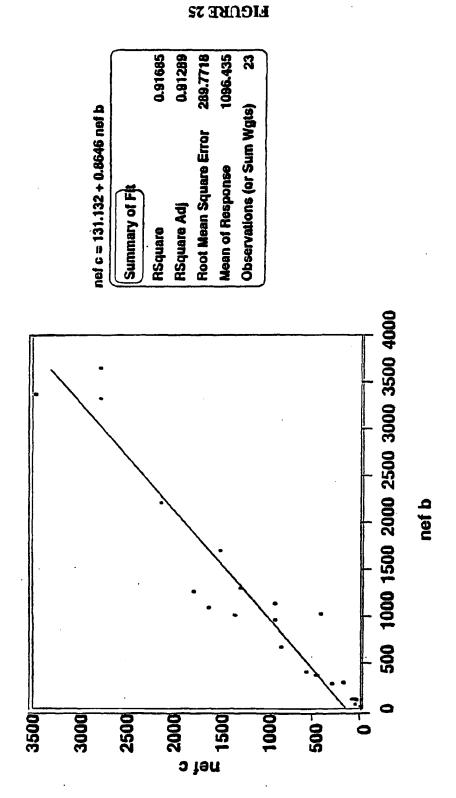


FIGURE 23

Comparison of Clade B vs. Clade C Anti-gag T Cell Responses in Clade B HIV-Infected Subjects



Comparison of Clade B vs. Clade C Anti-nef T Cell Responses in Clade B HIV-Infected Subjects



### MRKAd5pol MER1062 (MRKAd5 Pre-Adenoviral Vector Containing the IA opt pol Coding Region)

1 CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG GTAGTAGTTA TTATATGGAA TAAAACCTAA CTTCGGTTAT ACTATTACTC 51 GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG CCCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC CGCCCACTGC 101 TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT 151 GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG CGCTGCCTAC ACCGTTTTCA CTGCAAAAAC CACACGCGGC CACATGTGTC 201 GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTTGGG CTTCACTGTT AAAAGCGCGC CAAAATCCGC CTACAACATC ATTTAAACCC 251 CGTAACCGAG TAAGATTTGG CCATTTTCGC GGGAAAACTG AATAAGAGGA GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCCTTTTGAC TTATTCTCCT 301 AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA TCACTTTAGA CTTATTAAAA CACAATGAGT ATCGCGCATT ATAAACAGAT 351 GGGCCGCGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT CCCGGCGCCC CTGAAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA 401 CTCAGGTGTT TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG GAGTCCACAA AAGGCGCAAG GCCCAGTTTC AACCGCAAAA TAATAATATC 451 GCGGCCGCGA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT CGCCGGCGCT AGGTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA 501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC ATATAACCGA GTACAGGTTG TAATGGCGGT ACAACTGTAA CTAATAACTG 551 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT 601 TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC 651 CCCAACGACC CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA 701 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT TTGCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA 751 AAACTGCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC TTTGACGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG 801 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA GGATAACTGC AGTTACTGCC ATTTACCGGG CGGACCGTAA TACGGGTCAT 851 CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA GTACTGGAAT ACCCTGAAAG GATGAACCGT CATGTAGATG CATAATCAGT

7 i jure 26A

901	AGCGATAATG	GTACCACTAC	GCCAAAACCG	AGTACATCAA TCATGTAGTT	ACCCGCACCT
<b>95</b> 1	ATCGCCAAAC	TGAGTGCCCC	TAAAGGTTCA	CTCCACCCCA GAGGTGGGGT	AACTGCAGTT
1001		AAAACCGTGG	TTTTAGTTGC	CCTGAAAGGT	TTTACAGCAT
1051	TGTTGAGGCG	GGGTAACTGC	GTTTACCCGC	GTAGGCGTGT CATCCGCACA	TGCCACCCTC
1101	CAGATATATT	CGTCTCGAGC	AAATCACTTG	CGTCAGATCG GCAGTCTAGC	GGACCTCTGC
1151	GGTAGGTGCG	ACAAAACTGG	AGGTATCTTC	ACACCGGGAC TGTGGCCCTG	GCTAGGTCGG
1201	AGGCGCCGGC	CCTTGCCACG	TAACCTTGCG	GGATTCCCCG CCTAAGGGGC	ACGGTTCTCA
1251	CTCTAGATGG	TACCGGGGGT	AGAGGGGGTA	TGAGACTGTG ACTCTGACAC	GGACACTTCG
1301	ACTTCGGACC	GTACCTACCG	GGGTTCCACT	AGCAGTGGCC TCGTCACCGG	GGACTGACTC
1351	CTCTTCTAGT	TCCGGGACCA	CCTTTAGACG	ACTGAGATGG TGACTCTACC	TCTTCCTCCC
1401	GTTTTAGAGG	TTCTAACCGG	GGCTCTTGGG	CTACAACACC GATGTTGTGG	GGACACAAAC
1451	GGTAGTTCTT	CTTCCTGAGG	TGGTTCACCT	GGAAGCTGGT CCTTCGACCA	CCTGAAGTCC
1501	CTCGACTTGT	TCTCCTGGGT	CCTGAAGACC	GAGGTGCAGC CTCCACGTCG	ACCCGTAGGG
1551	GGTGGGGCGA	CCGGACTTCT	TCTTCTTCAG	TGTGACTGTG ACACTGACAC	GACCGACACC
1601	CCCTACGGAT	GAAGAGACAC	GGGGACCTAC	AGGACTTCAG TCCTGAAGTC	CTTCATGTGA
٠		AGGGGAGGTA	GTTGTTACTC	TGGGGACCGT	AGTCCATGGT
		GACGGGGTCC	CGACCTTCCC	GAGGGGACGG	TAGAAGGTCA
		GTTCTAGGAC	CTCGGGAAGT	CCTTCGTCTT	GGGACTGTAA
1801	GTGATCTACC CACTAGATGG	AGTACATGGC TCATGTACCG	TGCCCTGTAT	GTGGGCTCTG CACCCGAGAC	ACCTGGAGAT TGGACCTCTA

Figure 26B

1851	TGGGCAGCAC ACCCGTCGTG	A CCAAGA TCCTGGTTCT	TTGAGGAGCT AACTCCTCGA	GAGGCAGCAC CTCCGTCGTG	CTGCTG T GACGACTCCA
1901	GGGGCCTGAC CCCCGGACTG	CACCCCTGAC GTGGGGACTG	AAGAAGCACC TTCTTCGTGG	AGAAGGAGCC TCTTCCTCGG	CCCCTTCCTG GGGGAAGGAC
1951	TGGATGGGCT ACCTACCCGA		CCCCGACAAG GGGGCTGTTC		
2001	GCTGCCTGAG CGACGGACTC	AAGGACTCCT TTCCTGAGGA	GGACTGTGAA CCTGACACTT	TGACATCCAG ACTGTAGGTC	AAGCTGGTGG TTCGACCACC
2051		GACCCGGAGG	GTTTAGATGG	GACCGTAGTT	CCACTCCGTC
2101	GACACGTTCG	ACGACTCCCC		GACTGACTCC	ACTAGGGGGA
2151	CTGACTCCTC	CGACTCGACC	AGCTGGCTGA TCGACCGACT	CTTGTCCCTC	TAGGACTTCC
2201	TCGGACACGT	ACCCCACATG	TATGACCCCT ATACTGGGGA	GGTTCCTGGA	CTAACGACTC
2251	TAGGTCTTCG	TCCCGGTCCC	CCAGTGGACC GGTCACCTGG	ATGGTTTAGA	TGGTCCTCGG
2301	GAAGTTCTTG	GACTTCTGAC	CGTTCATACG	GTCCTACTCC	
2351	GGTTACTACA	CTTCGTCGAC	-TGACTCCGAC	ACCTOTTOTA	CACCACTGAG -GTGGTGACTC
2401	AGGTAACACT	AGACCCCGTT	CTGGGGGTTC	AAGTTCGACG	
2451	CCTCTGGACC	CTCTGGACCA	CCTGACTCAT	GACCGTCCGG	ACCTGGATCC TGGACCTAGG
2501	GACTCACCCT	CAAACACTIG	TGGGGGGGG	ACCACTTCGA	GTGGTACCAG CACCATGGTC
2551	GACCTCTTCC	TCGGGTAACA	CCCCCGACTC	TGGAAGATAC	TGGCTGGGGC ACCGACCCCG
2601	ACGGTTGTCC	CTCTGGTTCG	ACCCGTTCCG	ACCGATACAC	ACCAACAGGG TGGTTGTCCC
2651	GCAGGCAGAA CGTCCGTCTT	GGTGGTGACC	CTGACTGACA GACTGACTGT	CCACCAACCA	GAAGACTGCC
2701	CTCCAGGCCA GAGGTCCGGT	TCTACCTGGC AGATGGACCG	CCTCCAGGAC GGAGGTCCTG	TCTGGCCTGG AGACCGGACC	AGGTGAACAT TCCACTTGTA
2751	TGTGACTGCC ACACTGACGG	TCCCAGTATG AGGGTCATAC	CCCTGGGCAT	CATCCAGGCC GTAGGTCCGG	CAGCCTGATC GTCGGACTAG

Figure 26 C

2801	AGTCTGAGTC TCAGACTCAG	T CTGGTG ACTCGACCAC	AACCAGÁTCA TTGGTCTAGT	TTGAGCAGCT AACTCGTCGA	GATCAA G CTAGTTC TC
2851	GAGAAGGTGT CTCTTCCACA	ACCTGGCCTG TGGACCGGAC	GGTGCCTGCC CCACGGACGG	CACAAGGGCA GTGTTCCCGT	TTGGGGGCAA AACCCCCGTT
2901		GACAAGCTGG CTGTTCGACC			
2951	TGGATGGCAT ACCTACCGTA	TGACAAGGCC ACTGTTCCGG	CAGGATGAGC GTCCTACTCG	ATGAGAAGTA TACTCTTCAT	CCACTCCAAC GGTGAGGTTG
3001	TGGAGGGCTA ACCTCCCGAT	TGGCCTCTGA ACCGGAGACT	CTTCAACCTG GAAGTTGGAC	CCCCTGTGG GGGGGACACC	TGGCTAAGGA ACCGATTCCT
3051	CTAACACCGG	TCCTGTGACA AGGACACTGT	TCACGGTCGA	CTTCCCCCTC	CGGTACGTAC
3101	CCGTCCACCT	CTGCTCCCCT GACGAGGGGA	CCGTAGACCG	TCGACCGGAC	GTGGGTGGAC
3151	CTCCCGTTCC	TGATCCTGGT ACTAGGACCA	CCGACACGTA	CACCGGAGGC	CGATGTAACT
3201	CCGACTCCAC	ATCCCTGCTG TAGGGACGAC	TCTGTCCGGT	CCTCTGACGG	ATGAAGGACG
3251	ACTTCGACCG	TGGCAGGTGG ACCGTCCACC	GGACACTTCT	GGTAGGTGTG	ACGGTTACCG
3301	AGGTTGAAGT	CTGGGGCCAC GACCCCGGTG	TCACTCCCGA	CGGACGACCA	CCCGACCGTA
3351	GTTCGTCCTC	TTTGGCATCC AAACCGTAGG	GGATGTTGGG	GGTCAGGGTC	CCCCACCACC
3401	GGAGGTACTT	CAAGGAGCTG GTTCCTCGAC	TTCTTCTAGT	AACCCGTCCA	CTCCCTGGTC
3451		TGAAGACAGC ACTTCTGTCG			
3501	GITCICCITC	GGGGGCATCG	CCCCGATGAG	GCGACCCCTC	TCCTAACACC
		GTGTCTGTAG	GTCTGGTTCC	TCGAGGTCTT	CGTCTAGTGG
		TGAAGTCCCA	CATGATGTCC	CTGAGGTCCT	TGGGGGACAC
3651	GAAGGGCCCT CTTCCCGGGA	GCCAAGCTGC CGGTTCGACG	TGTGGAAGGG ACACCTTCCC	GGAGGGGGCT	GTGGTGATCC CACCACTAGG
3701	AGGACAACTC TCCTGTTGAG	TGACATCAAG ACTGTAGTTC	GTGGTGCCCA CACCACGGGT	GGAGGAAGGC CCTCCTTCCG	CAAGATCATC GTTCTAGTAG

Figure 26 D

3751	AGGGACTATG TCCCTGATAC	AGCAGAT CC. TCGTCTA	GGCTGGGGAT CCGACCCCTA	GACTGTGTGG CTGACACACC	CCTCCA CA GGAGGT GT
					> CTTTCCC> CC
3801	GGATGAGGAC	TAAAGCCCGG	GCAGATCTGC CGTCTAGACG	TGTGCCTTCT	AG11GCCAGC
	CCTACTCCTG	ATTICGGGCC	CGTCTAGACG	ACACGGAAGA	TCHACGGICG
3851	CATCTGTTGT	TTGCCCCTCC	CCCGTGCCTT	CCTTGACCCT	GGAAGGTGCC
3031	GTAGACAACA	AACGGGGAGG	GGGCACGGAA	GGAACTGGGA	CCTTCCACGG
3901			ATAAAATGAG		
	TGAGGGTGAC	AGGAAAGGAT	TATTTTACTC	CTTTAACGTA	GCGTAACAGA
2051	GAGTAGGTGT	C 2 mm-m 2 mm-c	TOCCOCCTOC	сстсссссъс	GACAGCAAGG
3931	CACIACGICI	GTANGATANG	ACCCCCACC	CCACCCCGTC	CTGTCGTTCC
4001	GGGAGGATTG	GGAAGACAAT	AGCAGGCATG	CTGGGGATGC	CCTCCCCTCT
	CCCTCCTAAC	CCTTCTGTTA	TCGTCCGTAC	GACCCCTACG	CCACCCGAGA
				~~~~~~~	
4051	ATGGCCGATC	GGCGCGCCGT	ACTGAAATGT TGACTTTACA	CACCCCCACC	CHARGGGIG
	TACCGGCTAG	CCGCGCGGCA	1GACTITACA	CACCCGCACC	Grafficente
4101	GGAAAGAATA	TATAAGGTGG	GGGTCTTATG	TAGTTTTGTA	TCTGTTTTGC
	CCTTTCTTAT	ATATTCCACC	CCCAGAATAC	ATCAAAACAT	AGACAAAACG
4151	AGCAGCCGCC	GCCGCCATGA	GCACCAACTC	GTTTGATGGA	AGCATTGTGA
	TCGTCGGCGG	CGGCGGTACT	CGTGGTTGAG	CAAACTACCT	TCGTAACACT
4201	GCTCATATTT	CACAACGCGC	<b>ATRICCOCCAT</b>	CCCCCCGGT	GCGTCAGAAT
4201	CGAGTATAAA	CTGTTGCGCG	TACGGGGGTA	CCCGGCCCCA	CGCAGTCTTA
4251	GTGATGGGCT	CCAGCATTGA	TGGTCGCCCC	GTCCTGCCCG	CAAACTCTAC
	CACTACCCGA	GGTCGTAACT	ACCAGCGGGG	CAGGACGGGC	GTTTGAGATG _
			B0808003330	OCCOMBCC & C	ACTGCAGCCT
4301	TACCTTGACC	TACGAGACCG	TGTCTGGAAC	CCCCTTGGAG	TGACGTCGGA
	ATGGAACTGG	ATGUTUTGGC	ACAGACC116	CGGCMCCIC	101001001
4351	CCCCCCCCC	TTCAGCCGCT	GCAGCEACCG	CCCGCGGGAT	TGTGACTGAC
	GCCGCCGCG	AAGTCGGCGA	CGTCGGTGGC	GGGCGCCCTA	ACACTGACTG
4401	TTTGCTTTCC	TGAGCCCGCT	TGCAAACAGT	GCAGCTTCCC	GTTCATCCGC
	AAACGAAAGG	ACTCGGGCGA	. ACGTTTGTCA	CGTCGAAGGG	CAAGTAGGCG
4451	CCCCCATGAC	AACTTCACGG	CTCTTTTGGC	ACAATTGGAT	TCTTTGACCC
4431	GGCGCTACTG	TTCAACTGCC	GAGAAAACCG	TGTTAACCTA	AGAAACTGGG
4501	GGGAACTTAA	TGTCGTTTCT	CAGCAGCTGT	TGGATCTGCG	CCAGCAGGTT
	CCCTTGAATT	ACAGCAAAGA	CTCGTCGACA	ACCTAGACGO	GGTCGTCCAA
		> 000mmC@ffC	י כער בתערער בי את	י כרכפיידיים א	ACATAAATAA
4551	TCTGCCCTGA NCNCGCGACT	TOCCARGGAG	GGGAGGGTTA	CGCCAAATTI	TGTATTTATT
4601	AAAACCAGAC	TCTGTTTGGA	TTTGGATCAA	GCAAGTGTCT	TGCTGTCTTT
	TTTTGGTCTG	AGACAAACCI	AAACCTAGTI	CGTTCACAG	ACGACAGAAA
4651	ATTTAGGGGT	TTTGCGCGCG	CGGTAGGCCC	GGGACCAGCG	CAGAGCCAGC
	TAAATCCCCA	AAAUGUGUGU	. GCCATCCGGC	,	, cagascasse

Figure 26E

4701	TTGAGGGTCC AACTCCCAGG	TCTGTATTTT ATAAAA	TTCCAGGACG AAGGTCCTGC	TGGTAAAGGT ACCATTTCCA	-GACTCPCGAT CTGAGA A
4751				GGGGTGGAGG CCCCACCTCC	
4801	GCAGAGCTTC CGTCTCGAAG			AGATGATCCA TCTACTAGGT	
4851				TTCAGTAGCA AAGTCATCGT	
4901	CAGGGGCAGG GTCCCCGTCC	CCCTTGGTGT GGGAACCACA	AAGTGTTTAC TTCACAAATG	AAAGCGGTTA TTTCGCCAAT	AGCTGGGATG TCGACCCTAC
4951	CCACGTATGC	ACCCCTATAC	TCTACGTAGA	TGGACTGTAT ACCTGACATA	AAAATCCAAC
5001	CGATACAAGG	GTCGGTATAG	GGAGGCCCCT	TTCATGTTGT AAGTACAACA	CGTCTTGGTG
5051	GTCGTGTCAC	ATAGGCCACG	TGAACCCTTT	TTTGTCATGT AAACAGTACA	TCGAATCTTC
5101				TGTGACCTCC ACACTGGAGG	
5151	TACGTAAGCA	GGTATTACTA	CCGTTACCCG	CCACGGGCGG GGTGCCCGCC	GCCGGACCCG
5201	CTTCTATAAA	GACCCTAGTG	ATTGCAGTAT	GTTGTGTTCC CAACACAAGG	TCCTACTCTA
5251	GCAGTATCCG	GTAAAAATGT	TTCGCGCCCG	GGAGGGTGCC CCTCCCACGG	TCTGACGCCA
5301	TATTACCAAG	GTAGGCCGGG	TCCCCGCATC	TTACCCTCAC AATGGGAGTG	TCTAAACGTA
5351	AAGGGTGCGA	AACTCAAGTC	TACCCCCCTA	CATGTCTACC GTACAGATGG	ACGCCCCGCT
5401	TGAAGAAAAC ACTTCTTTTG	GGTTTCCGGG CCAAAGGCCC	GTAGGGGAGA CATCCCCTCT	TCAGCTGGGA AGTCGACCCT	AGAAAGCAGG TCTTTCGTCC
5451	AAGGACTCGT	CGACGCTGAA	TGGCGTCGGC	CACCCGGGCA	
		ACGTTGACCA	TCAATTCTCT	CGACGTCGAC	GGCAGTAGGG
		CCGGTGAAGC	AATTCGTACA	GGGACTGAGC	GTACAAAAGG
5601	CTGACCAAAT GACTGGTTTA	CCGCCAGAAG GGCGGTCTTC	CCCCTCCCCC	CCCAGCGATA GGGTCGCTAT	GCAGTTCTTG CGTCAAGAAC

Figure 26F

5651	CAAGGAAGCA GTTCCTTCGT			ACCGTCCGCC TGGCAGGCGG	
5701				GGTCCCACAG CCAGGGTGTC	CTCGGTCACC GAGCCAGTGG
5751	TGCTCTACGG ACGAGATGCC			CCTCGTTTCG GGAGCAAAGC	
5801					GGGCCAGGGT CCCGGTCCCA
5851	CATGTCTTTC GTACAGAAAG			CAGCGTAGTC GTCGCATCAG	
5901	ACTTCCCCAC	GCGAGGCCCG	ACGCGCGACC	GGTCCCACGC	
5951	CAGGACGACC	ACGACTTCGC	GACGGCCAGA	AGCGGGACGC	•
6001	CATCGTAAAC	TGGTACCACA	GTATCAGGTC	GGGGAGGCGC	GCGTGGCCCT
6051	ACCGCGCGTC	GAACGGGAAC	CTCCTCCGCG	GCGTGCTCCC	
6101	CTTTTGAGGG GAAAACTCCC	CGTAGAGCTT GCATCTCGAA	GGGCGCGAGA CCCGCGCTCT	AATACCGATT TTATGGCTAA	CCGGGGAGTA GGCCCCTCAT
6151				CTCGCATTCC GAGCGTAAGG	ACGAGCCAGG TGCTCGGTCC
6201				GGTTTCCCCC CCAAAGGGGG	ATGCTTTTTG TACGAAAAAC
6251					GCTCGGTGAC CGAGCCACTG
6301	CTTTTCCGAC	AGGCACAGGG	GCATATGTCT	GAACTCTCCG	CTGTCCTCGA GACAGGAGCT
	CGCCACAAGG	CGCCAGGAGG	AGCATATCTT	TGAGCCTGGT	CTCTGAGACA GAGACTCTGT
6401	AAGGCTCGCG TTCCGAGCGC				AGGGGTAGCG TCCCCATCGC
6451					AGACACATGT TCTGTGTACA
	GCGGGAGAAG	CCGTAGTTCC	TTCCACTAAC	CAAACATCCA	GTAGGCCACG CATCCGGTGC
6551					GGGCGCGTTC CCCGCGCAAG

Figure 266

6601	GTCCTCACTC CAGGAGTGAG	TCTTCCGCAT AGGCGTA	CGCTGTCTGC GCGACAGACG	CTCCCGGTCG	ACAACO C
6651	AGTACTCCCT	CTGAAAAGCG	GGCATGACTT	CTGCGCTAAG	ATTGTCAGTT
	TCATGAGGGA	GACTTTTCGC	CCGTACTGAA	GACGCGATTC	TAACAGTCAA
6701	TCCAAAAACG	AGGAGGATTT	GATATTCACC	TGGCCCGCGG	TGATGCCTTT
	AGGTTTTTGC	TCCTCCTAAA	CTATAAGTGG	ACCGGGCGCC	ACTACGGAAA
6751	GAGGGTGGCC	GCATCCATCT	GGTCAGAAAA	GACAATCTTT	TTGTTGTCAA
	CTCCCACCGG	CGTAGGTAGA	CCAGTCTTTT	CTGTTAGAAA	AACAACAGTT
6801	GCTTGGTGGC	AAACGACCCG	TAGAGGGCGT	TGGACAGCAA	CTTGGCGATG
	CGAACCACCG	TTTGCTGGGC	ATCTCCCGCA	ACCTGTCGTT	GAACCGCTAC
6851	CTCGCGTCCC	AAACCAAAAA	CAGCGCTAGC	GCGCGCTCCT CGCGCGAGGA	ACCGGCGCTA
6901	GTTTAGCTGC	ACGTATTCGC	GCGCAACGCA	CCGCCATTCG	GGAAAGACGG
	CAAATCGACG	TGCATAAGCG	CGCGTTGCGT	GGCGGTAAGC	CCTTTCTGCC
6951	TGGTGCGCTC	GTCGGGCACC	AGGTGCACGC	GCCAACCGCG	GTTGTGCAGG
	ACCACGCGAG	CAGCCCGTGG	TCCACGTGCG	CGGTTGGCGC	CAACACGTCC
7001	GTGACAAGGT	CAACGCTGGT	GGCTACCTCT	CCGCGTAGGC	GCTCGTTGGT
	CACTGTTCCA	GTTGCGACCA	CCGATGGAGA	GGCGCATCCG	CGAGCAACCA
7051	CCAGCAGAGG	CGGCCGCCCT	TGCGCGAGCA	GAATGGCGGT	AGGGGGTCTA
	GGTCGTCTCC	GCCGGCGGGA	ACGCGCTCGT	CTTACCGCCA	TCCCCCAGAT
7101	GCTGCGTCTC	GTCCGGGGGG	TCTGCGTCCA	CGGTAAAGAC	CCCGGGCAGC
	CGACGCAGAG	CAGGCCCCCC	AGACGCAGGT	GCCATTTCTG	GGGCCCGTCG
7151	AGGCGCGCGT	CGAAGTAGTC	TATCTTGCAT	CCTTGCAAGT	CTAGCGCCTG
	TCCGCGCGCA	GCTTCATCAG	ATAGAACGTA	GGAACGTTCA	GATCGCGGAC
7201	CTGCCATGCG GACGGTACGC	CGGGCGGCAA	GCGCGCGCTC	GTATGGGTTG CATACCCAAC	AGTGGGGGAC TCACCCCTG
7251	CCCATGGCAT	GGGGTGGGTG	AGCGCGGAGG	CGTACATGCC	GCAAATGTCG
	GGGTACCGTA	CCCCACCCAC	TCGCGCCTCC	GCATGTACGG	CGTTTACAGC
7301	TAAACGTAGA	GGGGCTCTCT	GAGTATTCCA	AGATATGTAG	GGTAGCATCT
	ATTTGCATCT	CCCCGAGAGA	CTCATAAGGT	TCTATACATC	CCATCGTAGA
7351	TCCACCGCGG	ATGCTGGCGC	GCACGTAATC	GTATAGTTCG	TGCGAGGGAG
	AGGTGGCGCC	TACGACCGCG	CGTGCATTAG	CATATCAAGC	ACGCTCCCTC
7401	CGAGGAGGTC	GGGACCGAGG	TTGCTACGGG	CGGGCTGCTC	TGCTCGGAAG
	GCTCCTCCAG	CCCTGGCTCC	AACGATGCCC	GCCCGACGAG	ACGAGCCTTC
7451	ACTATCTGCC	TGAAGATGGC	ATGTGAGTTG	GATGATATGG	TTGGACGCTG
	TGATAGACGG	ACTTCTACCG	TACACTCAAC	CTACTATACC	AACCTGCGAC
7501	GAAGACGTTG	AAGCTGGCGT	CTGTGAGACC	TACCGCGTCA	CGCACGAAGG
	CTTCTGCAAC	TTCGACCGCA	GACACTCTGG	ATGGCGCAGT	GCGTGCTTCC

Figure 26 H

7551	AGGCGTAGGA TCCGCATCCT	CAGCGCGTCG	TTGTTGACCA AACAACTGGT	GCTCGGCGGT CGAGCCGCCA	GACCTG G CTGGACGTGC
7601	TCTAGGGCGC AGATCCCGCG	AGTAGTCCAG TCATCAGGTC	GGTTTCCTTG CCAAAGGAAC	ATGATGTCAT TACTACAGTA	ACTTATCCTG TGAATAGGAC
7651	TCCCTTTTTT AGGGAAAAAA	TTCCACAGCT AAGGTGTCGA	CGCGGTTGAG GCGCCAACTC	GACAAACTCT CTGTTTGAGA	TCGCGGTCTT AGCGCCAGAA
7701	AGGTCATGAG	TTGGATCGGA AACCTAGCCT	TTGGGCAGCC	GGAGGCTTGC	CATTCTCGGA
7751	TCGTACATCT	ACTGGTTGAC TGACCAACTG	CCGGACCATC	CGCGTCGTAG	GGAAAAGATG
7801	CCCATCGCGC	TATGCCTGCG ATACGGACGC	GCCGGAAGGC	CTCGCTCCAC	ACCCACTCGC
7851	GTTTCCACAG	CCTGACCATG GGACTGGTAC	TGAAACTCCA	TGACCATAAA	CTTCAGTCAC
7901	AGCAGCGTAG	CGCCCTGCTC GCGGGACGAG	GGTCTCGTTT	TTCAGGCACG	CGAAAAACCT
7951	TGCGCCTAAA	GGCAGGGCGA CCGTCCCGCT	TCCACTGTAG	CAACTTCTCA	TAGAAAGGGC
8001	GCGCTCCGTA	AAAGTTGCGT TTTCAACGCA	CACTACGCCT	TCCCAGGGCC	GTGGAGCCTT
8051	GCCAACAATT		CCGCTCGTGC	TAGAGCAGTT	TCGGCAACTA
8101	CAACACCGGG	ACAATGTAAA TGTTACATTT	CAAGGTTCTT	CGCGCCCTAC	GGGAACTACC
8151	TTCCGTTAAA	AAATTCAAGG	AGCATCCACT	CGAGAAGTCC	GGAGCTGAGC
8201	GGCACGAGAC	TTTCCCGGGT	CAGACGTTCT	ACTCCCAACC	AAGCGACGAA
8251	ACTCGAGGTG	TCCAGTGCCC	GGTAATCGTA	AACGTCCACC	TCGCGAAAGG AGCGCTTTCC
8301	AGGATTTGAC	CGCTGGATAC	CGGTAAAAA	GACCCCACTA	GCAGTAGAAG CGTCATCTTC
8351	GTAAGCGGGT CATTCGCCCA	CTTGTTCCCA GAACAAGGGT	GCGGTCCCAT CGCCAGGGTA	CCAAGGTTCG GGTTCCAAGG	CGGCTAGGTC CGCGATCCAG
8401	TCGCGCGGCA AGCGCGCCGT	GTCACTAGAG CAGTGATCTC	GCTCATCTCC CGAGTAGAGG	GCCGAACTTC GCCTTGAAC	ATGACCAGCA TACTGGTCGT
8451	TGAAGGGCAC ACTTCCCGTG	GAGCTGCTTC CTCGACGAAG	CCAAAGGCCC GGTTTCCGGG	CCATCCAAG GGTAGGTTC	TATAGGTCTCT A TATCCAGAGA

Figure 26I

8501	ACATCGTAGG TGTAGCATCC	TAAAGAG ACTGTTTCTC	ACGCTCGGTG TGCGAGCCAC	CGAGGATGCG GCTCCTACGC	AGCCGA GG TCGGCTAGCC
8551	GAAGAACTGG CTTCTTGACC	ATCTCCCGCC TAGAGGGCGG	ACCAATTGGA TGGTTAACCT	GGAGTGGCTA CCTCACCGAT	TTGATGTGGT AACTACACCA
8601	GAAAGTAGAA CTTTCATCTT	GTCCCTGCGA CAGGGACGCT	CGGGCCGAAC GCCCGGCTTG	ACTCGTGCTG TGAGCACGAC	GCTTTTGTAA CGAAAACATT
8651	TTTGCACGCG	TCATGACCGT	CGCCACGTGC	GGCTGTACAT CCGACATGTA	GGACGTGCTC
8701	CAACTGGACT	GCTGGCGCGT	GTTCCTTCGT	GAGTGGGAAT CTCACCCTTA	AACTCGGGGA
8751	GCGGACCGCC	CAAACCGACC	ACCAGAAGAT	CTTCGGCTGC GAAGCCGACG	AACAGGAACT
8801	GGCAGACCGA	CGAGCTCCCC	TCAATGCCAC	GATCGGACCA CTAGCCTGGT	GGTGCGGCGC
8851	GCTCGGGTTT	CAGGTCTACA	GGCGCGCGCC	CGGTCGGAGC GCCAGCCTCG	AACTACTGTT
8901	GTAGCGCGTC	TACCCTCGAC	AGGTACCAGA	GGAGCTCCCG CCTCGAGGGC	GCCGCAGTCC
8951	AGTCCGCCCT	CGAGGACGTC	CAAATGGAGC	CATAGACGGG GTATCTGCCC	AGTCCCGCGC
9001	CCGATCTAGG	TCCACTATGG	ATTAAAGGTC	GGGCTGGTTG CCCGACCAAC	CACCGCCGCA
9051	GCTACCGAAC	GTTCTCCGGC	GTAGGGGCGC	GCGCGACTAC CGCGCTGATG	CCATGGCGCG
9101	CCGCCCGCCA	CCCGGCGCCC	CCACAGGAAC	GATGATGCAT CTACTACGTA	GATTTTCGCC
9151	TGACGCGGGC ACTGCGCCCG	GAGCCCCCGG CTCGGGGGCC	AGGTAGGGGG TCCATCCCCC	GGCTCCGGAC CCGAGGCCTG	CCGCCGGGAG GGCGGCCCTC
9201	TCCCCCGTCC	CCGTGCAGCC	GCGGCGCGCG	GGGCAGGAGC	ACCACGACGC
9251		CGACCGCTTG	CGCTGCTGCG	CCGCCAACTA	GAGGACTTAG
9301	TGGCGCCTCT ACCGCGGAGA	GCGTGAAGAC CGCACTTCTG	GACGGGCCCG CTGCCCGGCC	GTGAGCTTGA CACTCGAACT	ACCTGAAAGA TGGACTTTCT
9351	GAGTTCGACA CTCAAGCTGT	GAATCAATTT CTTAGTTAAA	CGGTGTCGTT GCCACAGCAA	CACGGCGGCC	TGGCGCAAAA ACCGCGTTTT
9401	TCTCCTGCAC AGAGGACGTG	GTCTCCTGAG CAGAGGACTC	TTGTCTTGAT AACAGAACTA	AGGCGATCTC TCCGCTAGAG	GGCCATGAAC CCGGTACTTG

Figure 26 J

9451	TGCTCGATCT ACGAGCTAGA			CGTCCGGCTC GCAGGCCGAG	
9501				GAGCTGCGAG CTCGACGCTC	
9551	GGCCTCCCTC CCGGAGGGAG			CCACGCCCCC	
9601				AGCTCCACGT TCGAGGTGCA	
9651	CTGCCGCATC	AAAGCGTCCG	CGACTTTCTC	GTAGTTGAGG CATCAACTCC	CACCACCGCC
9701				AGCGTCGCAA TCGCAGCGTT	
9751	AACTATAGGG	GGTTCCGGAG	TTCCGCGAGG	ATGGCCTCGT TACCGGAGCA	TCTTCAGGTG
9801				CGACACGGTT GCTGTGCCAA	
9851	GGTCTTCTGC	CTACTCGAGC	CGCTGTCACA	CGCGCACCTC GCGCGTGGAG	CGCGAGTTTC
9901				TCCTCTTCCA AGGAGAAGGT	
9951	CCCTTCTTCT GGGAAGAAGA	TCTTCTGGCG AGAAGACCGC	GCGGTGGGGG	AGGGGGGACA TCCCCCTGT	CGGCGGCGAC GCCGCCGCTG -
10001	CTGCCGCGTG	GCCCTCCGCC	AGCTGTTTCG	GCTCGATCAT CGAGCTAGTA	GAGGGGCGCC
10051	GCTGCCGCGT	ACCAGAGCCA	CTGCCGCGCC	CCGTTCTCGC GGCAAGAGCG	CCCCCGCGTC
10101	AACCTTCTGC	GGCGGGCAGT	ACAGGGCCAA	ATGGGTTGGC TACCCAACCG	CCCCCGACG
10151	GTACGCCGTC	CCTATGCCGC	GATTGCTACG	TAGAGTTGTT	TTGTTGTGTA AACAACACAT
,		CCCCCTCCCT	GGACTCGCTC	AGGCGTAGCT	GGCCTAGCCT
	TTTGGAGAGC	TCTTTCCGCA	GATTGGTCAG	TGTCAGCGTT	GGTAGGCTGA CCATCCGACT
	CGTGGCACCG	CCCGCCGTCG	CCCGCCGCCA	GCCCCAACAA	TCTGGCGGAG AGACCGCCTC
10351	GTGCTGCTGA CACGACGACT	TGATGTAATT ACTACATTAA	AAAGTAGGCG TTTCATCCGC	GTCTTGAGAC CAGAACTCTG	GGCGGATGGT CCGCCTACCA

Figure 26 K

10401	CGACAGAAGC	A TGTCCT	TGGGTCCGGC	CTGCTGAATG	CCCAGG T
	GCTGTCTTCG	TACAGGA	ACCCAGGCCG	GACGACTTAC	GCGTCC A
10451	CGGCCATGCC	CCAGGCTTCG	TTTTGACATC	GGCGCAGGTC	TTTGTAGTAG
	GCCGGTACGG	GGTCCGAAGC	AAAACTGTAG	CCGCGTCCAG	AAACATCATC
10501				TCTTCTCCTT AGAAGAGGAA	
10551	TGCATCTCTT	GCATCTATCG	CTGCGGCGGC	GGCGGAGTTT	GGCCGTAGGT
	ACGTAGAGAA	CGTAGATAGC	GACGCCGCCG	CCGCCTCAAA	CCGGCATCCA
10601	GGCGCCCTCT	TCCTCCCATG	CGTGTGACCC	CGAAGCCCCT	CATCGGCTGA
	CCGCGGGAGA	AGGAGGGTAC	GCACACTGGG	GCTTCGGGGA	GTAGCCGACT
10651	AGCAGGGCTA	GCTCGGCGAC	AACGCGCTCG	GCTAATATGG	CCTGCTGCAC
	TCGTCCCGAT	CCAGCCGCTG	TTGCGCGAGC	CGATTATACC	GGACGACGTG
10701	CTGCGTGAGG	GTAGACTGGA	AGTCATCCAT	GTCCACAAAG	CGGTGGTATG
	GACGCACTCC	CATCTGACCT	TCAGTAGGTA	CAGGTGTTTC	GCCACCATAC
10751	GCGGGCACAA	CTACCACATT	CACGTCAACC	CCATAACGGA GGTATTGCCT	GGTCAATTGC
10801	GTCTGGTGAC	CCGGCTGCGA	GAGCTCGGTG	TACCTGAGAC	GCGAGTAAGC
	CAGACCACTG	GGCCGACGCT	CTCGAGCCAC	ATGGACTCTG	CGCTCATTCG
10851	CCTCGAGTCA	AATACGTAGT	CGTTGCAAGT	CCGCACCAGG	TACTGGTATC
	GGAGCTCAGT	TTATGCATCA	GCAACGTTCA	GGCGTGGTCC	ATGACCATAG
10901	CCACCAAAAA	GTGCGGCGGC	GGCTGGCGGT	AGAGGGGCCA	GCGTAGGGTG
	GGTGGTTTTT	CACGCCGCCG	CCGACCGCCA	TCTCCCCGGT	CGCATCCCAC
10951	GCCGGGGCTC CGGCCCCGAG	CGGGGGGGAG	ATCTTCCAAC TAGAAGGTTG	ATAAGGCGAT TATTCCGCTA	GATATCCGTA CTATAGGCAT
11001	GATGTACCTG CTACATGGAC	GACATCCAGG CTGTAGGTCC	TGATGCCGGC ACTACGGCCG	GCCGCCACCAC	GAGGCGCGCGC
11051	GAAAGTCGCG	GACGCGGTTC	CAGATGTTGC	GCAGCGGCAA	AAAGTGCTCC
	CTTTCAGCGC	CTGCGCCAAG	GTCTACAACG	CGTCGCCGTT	TTTCACGAGG
11101	ATGGTCGGGA	CGCTCTGGCC	GGTCAGGCGC	GCGCAATCGT	TGACGCTCTA
	TACCAGCCCT	GCGAGACCGG	CCAGTCCGCG	CGCGTTAGCA	ACTGCGAGAT
11151	GACCGTGCAA	AAGGAGAGCC	TGTAAGCGGG	CACTCTTCCG	TGGTCTGGTG
	CTGGCACGTT	TTCCTCTCGG	ACATTCGCCC	GTGAGAAGGC	ACCAGACCAC
11201	GATAAATTCG	CAAGGGTATC	ATGGCGGACG	ACCGGGGTTC	GAGCCCCGTA
	CTATTTAAGC	GTTCCCATAG	TACCGCCTGC	TGGCCCCAAG	CTCGGGGCAT
11251	TCCGGCCGTC AGGCCGGCAG	CGCCGTGATC GCGGCACTAG	CATGCGGTTA GTACGCCAAT	CCGCCGCGCA	GTCGAACCCA CAGCTTGGGT
11301	GGTGTGCGAC	GTCAGACAAC	GGGGGAGTGC	TCCTTTTGGC	TTCCTTCCAG
	CCACACGCTG	CAGTCTGTTG	CCCCTCACG	AGGAAAACCG	AAGGAAGGTC

Figure 26L

11351	CCCCCCCCCC	T TGCGCTA ACGACGCGAT	GCTTTTTTGG CGAAAAAACC	CCACTGGCCG GGTGACCGGC	CGCGCA TT GCGCGT CCA
11401	AAGCGGTTAG TTCGCCAATC			AGTGGCTCGC TCACCGAGCG	
11451				GGGACCCCCG CCCTGGGGGC	
11501				TTGCCTCCCC AACGGAGGGG	
11551				GACGAGCCCC CTGCTCGGGG	
11601				GCGCCCCCT CGCGGGGGA	
11651				GGGCACCCTC CCCGTGGGAG	
11701				GACGCGGCAG CTGCGCCGTC	CAGATGGTGA GTCTACCACT
11751				CTACCTGGAC GATGGACCTG	
11801				CTCCTGAGCG GAGGACTCGC	
11851				TACGTGCCGC ATGCACGGCG	
11901				GGAGATGCGG CCTCTACGCC	
11951				TGAATCGCGA ACTTAGCGCT	
12001				ACCGGGATTA TGGCCCTAAT	
12051				CGCATACGAG GCGTATGCTC	
12101	ACCAGGAGAT TGGTCCTCTA				GCGTACGCTT CGCATGCGAA
12151	GTGGCGCGCG CACCGCGCGC				GGGACTTTGT CCCTGAAACA
12201	AAGCGCGCTG TTCGCGCGAC				GCGCAGCTGT CGCGTCGACA
12251	TCCTTATAGT AGGAATATCA				

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12301	CTAAACATAG GATTTGTATC	ATTICGGGCT	CCCGGCGACC	GACGAGCTAA	ACTATTTTTA
12351	CCTGCAGAGC	ATAGTGGTGC	AGGAGCGCAG	CTTGAGCCTG	GCTGACAAGG
	GGACGTCTCG	TATCACCACG	TCCTCGCGTC	GAACTCGGAC	CGACTGTTCC
12401	TGGCCGCCAT	CAACTATTCC	ATGCTTAGCC	TGGGCAAGTT	TTACGCCCGC
	ACCGGCGGTA	GTTGATAAGG	TACGAATCGG	ACCCGTTCAA	AATGCGGGCG
12451	AAGATATACC	ATACCCCTTA	CGTTCCCATA	GACAAGGAGG	TAAAGATCGA
	TTCTATATGG	TATGGGGAAT	GCAAGGGTAT	CTGTTCCTCC	ATTTCTAGCT
12501	GGGGTTCTAC	ATGCGCATGG	CGCTGAAGGT	GCTTACCTTG	AGCGACGACC
	CCCCAAGATG	TACGCGTACC	GCGACTTCCA	CGAATGGAAC	TCGCTGCTGG
12551	TGGGCGTTTA	TCGCAACGAG	CGCATCCACA	AGGCCGTGAG	CGTGAGCCGG
	ACCCGCAAAT	AGCGTTGCTC	GCGTAGGTGT	TCCGGCACTC	GCACTCGGCC
12601	CGGCGCGAGC	TCAGCGACCG	CGAGCTGATG	CACAGCCTGC	AAAGGGCCCT
	GCCGCGCTCG	AGTCGCTGGC	GCTCGACTAC	GTGTCGGACG	TTTCCCGGGA
12651	GGCTGGCACG	GGCAGCGGCG	ATAGAGAGGC	CGAGTCCTAC	TTTGACGCGG
	CCGACCGTGC	CCGTCGCCGC	TATCTCTCCG	GCTCAGGATG	AAACTGCGCC
12701	GCGCTGACCT	GCGCTGGGCC	CCAAGCCGAC	GCGCCCTGGA	GCCAGCTGGG
	CGCGACTGGA	CGCGACCCGG	GGTTCGGCTG	CGCGGGACCT	CCGTCGACCC
12751	GCCGGACCTG CGGCCTGGAC	GGCTGGCGGT CCGACCGCCA	GGCACCCGCG	CGCGCTGGCA GCGCGACCGT	ACGTCGGCGG TGCAGCCGCC
12801	CGTGGAGGAA	TATGACGAGG	ACGATGAGTA	CGAGCCAGAG	GACGGCGAGT
	GCACCTCCTT	ATACTGCTCC	TGCTACTCAT	GCTCGGTCTC	CTGCCGCTCA
12851	ACTAAGCGGT	GATGTTTCTG	ATCAGATGAT	GCAAGACGCA	ACGGACCCGG
	TGATTCGCCA	CTACAAAGAC	TAGTCTACTA	CGTTCTGCGT	TGCCTGGGCC
12901	CGGTGCGGGC	GGCGCTGCAG CCGCGACGTC	AGCCAGCCGT TCGGTCGGCA	CCGGCCTTAA GGCCGGAATT	CTCCACGGAC GAGGTGCCTG
12951	GACTGGCGCC	AGGTCATGGA	CCGCATCATG	TCGCTGACTG	CGCGCAATCC
	CTGACCGCGG	TCCAGTACCT	GGCGTAGTAC	AGCGACTGAC	GCGCGTTAGG
13001	TGACGCGTTC	CGGCAGCAGC	CGCAGGCCAA	CCGGCTCTCC	GCAATTCTGG
	ACTGCGCAAG	GCCGTCGTCG	GCGTCCGGTT	GGCCGAGAGG	CGTTAAGACC
13051	AAGCGGTGGT TTCGCCACCA	GGGCCGCGCGC	GCAAACCCCA CGTTTGGGGT	CGCACGAGAA GCGTGCTCTT	GCTGCTGGCG
13101	ATCGTAAACG	CGCTGGCCGA	AAACAGGGCC	ATCCGGCCCG	ACGAGGCCGG
	TAGCATTTGC	GCGACCGGCT	TTTGTCCCGG	TAGGCCGGGC	TGCTCCGGCC
13151	CCTGGTCTAC	GACGCGCTGC	TTCAGCGCGT	GGCTCGTTAC	AACAGCGGCA
	GGACCAGATG	CTGCGCGACG	AAGTCGCGCA	CCGAGCAATG	TTGTCGCCGT
13201	ACGTGCAGAC	CAACCTGGAC	CGGCTGGTGG	GGGATGTGCG	CGAGGCCGTG
	TGCACGTCTG	GTTGGACCTG	GCCGACCACC	CCCTACACGC	GCTCCGGCAC

Figure 26 N.

13251			GCAGCAGGGC CGTCGTCCCG		
13301			CACAGCCCGC GTGTCGGGCG		
13351			AGCGCACTGC TCGCGTGACG		
13401			GTCTGGGCCA CAGACCCGGT	-	
13451			TAAACCTGAG ATTTGGACTC		
13501			GCTCCCACAG CGAGGGTGTC		
13551			GCGCCTGTTG CGCGGACAAC		
13601			CCCGGGACAC GGGCCCTGTG		
13651			GGTCAGGCGC CCAGTCCGCG		
13701			CCGCGCGCTG		
13751			ACCTGCTGAC TGGACGACTG		
13801	• •		AGCGAGGAGG TCGCTCCTCC		
13851			CCTGATGCGC GGACTACGCG		
13901			GCAACATGGA CGTTGTACCT		
13951	TGGCCGGCAA	ATAGTTGGCG	CTAATGGACT GATTACCTGA	TGAACGTAGC	GCGCCGGCGG
14001	CACTTGGGGC	TCATAAAGTG	GTTACGGTAG	AACTTGGGCG	ACTGGCTACC TGACCGATGG
14051	GCCCCTGGT	TTCTACACCG AAGATGTGGC	GGGGATTCGA CCCCTAAGCT	GGTGCCCGAG CCACGGGCTC	GGTAACGATG CCATTGCTAC
14101					GCAACCGCAG CGTTGGCGTC
14151					CGCTGCGAAA GCGACGCTTT

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14201	GGAAAGCTTC CCTTTCGAAG	CCTAGGCCAA GCCCGGTT	GCAGCTTGTC CGTCGAACAG	CGATCTAGGC* GCTAGATCCG	CGACGC G
14251	CGCGGTCAGA	TGCTAGTAGC	CCATTTCCAA	GCTTGATAGG	GTCTCTTACC
	GCGCCAGTCT	ACGATCATCG	GGTAAAGGTT	CGAACTATCC	CAGAGAATGG
14301	AGCACTCGCA	CCACCCGCCC	GCGCCTGCTG	GGCGAGGAGG	AGTACCTAAA
	TCGTGAGCGT	GGTGGGCGGG	CGCGGACGAC	CCGCTCCTCC	TCATGGATTT
14351	CAACTCGCTG	CTGCAGCCGC	AGCGCGAAAA	AAACCTGCCT	CCGGCATTTC
	GTTGAGCGAC	GACGTCGGCG	TCGCGCTTTT	TTTGGACGGA	GGCCGTAAAG
14401	CCAACAACGG	GATAGAGAGC	CTAGTGGACA	AGATGAGTAG	ATGGAAGACG
	GGTTGTTGCC	CTATCTCTCG	GATÇACCTGT	TCTACTCATC	TACCTTCTGC
14451			CGTGCCAGGC GCACGGTCCG		
14501	TCAAAGGCAC	GACCGTCAGC	GGGGTCTGGT	GTGGGAGGAC	GATGACTCGG
	AGTTTCCGTG	CTGGCAGTCG	CCCCAGACCA	CACCCTCCTG	CTACTGAGCC
14551	CAGACGACAG	CAGCGTCCTG	GATTTGGGAG	GGAGTGGCAA	CCCGTTTGCG
	GTCTGCTGTC	GTCGCAGGAC	CTAAACCCTC	CCTCACCGTT	GGGCAAACGC
14601	CACCTTCGCC	CCAGGCTGGG	GAGAATGTTT	TAAAAAAAA	AAAAGCATGA
	GTGGAAGCGG	GGTCCGACCC	CTCTTACAAA	TTTTTTTTT	TTTTCGTACT
14651	TGCAAAATAA	AAAACŢCACC	AAGGCCATGG	CACCGAGCGT	TGGTTTTCTT
	ACGTTTTATT	TTTTGAGTGG	TTCCGGTACC	GTGGCTCGCA	ACCAAAAGAA
14701	GTATTCCCCT	TAGTATGCGG	CGCGCGCCGA	TGTATGAGGA	AGGTCCTCCT
	CATAAGGGGA	ATCATACGCC	GCGCGCCGCT	ACATACTCCT	TCCAGGAGGA
14751	CCCTCCTACG GGGAGGATGC	AGAGTGTGGT TCTCACACCA	GAGCGCGGCG CTCGCGCCGC	CCAGTGGCGG GGTCACCGCC	CGGCGCGACCC
14801	TTCTCCCTTC	GATGCTCCCC	TGGACCCGCC	GTTTGTGCCT	CCGCGGTACC
	AAGAGGGAAG	CTACGAGGGG	ACCTGGGCGG	CAAACACGGA	GGCGCCATGG
14851	TGCGGCCTAC	CGGGGGGAGA	AACAGCATCC	GTTACTCTGA	GTTGGCACCC
	ACGCCGGATG	GCCCCCTCT	TTGTCGTAGG	CAATGAGACT	CAACCGTGGG
14901	CTATTCGACA	CCACCCGTGT	GTACCTGGTG	GACAACAAGT	CAACGGATGT
	GATAAGCTGT	GGTGGGCACA	CATGGACCAC	CTGTTGTTCA	GTTGCCTACA
14951	GGCATCCCTG	AACTACCAGA	ACGACCACAG	CAACTTTCTG	ACCACGGTCA
	CCGTAGGGAC	TTGATGGTCT	TGCTGGTGTC	GTTGAAAGAC	TGGTGCCAGT
15001	TTCAAAACAA AAGTTTTGTT	TGACTACAGC ACTGATGTCG	CCGGGGGAGG	CAAGCACACA GTTCGTGTGT	GACCATCAAT CTGGTAGTTA
15051	CTTGACGACC	GGTCGCACTG	GGGCGGCGAC	CTGAAAACCA	TCCTGCATAC
	GAACTGCTGG	CCAGCGTGAC	CCCGCCGCTG	GACTTTTGGT	AGGACGTATG
15101	CAACATGCCA	AATGTGAACG	AGTTCATGTT	TACCAATAAG	TTTAAGGCGC
	GTTGTACGGT	TTACACTTGC	TCAAGTACAA	ATGGTTATTC	AAATTCCGCG

Figure 26 P

15151		CAGCGCGAAC		
15201		TGGAGTTCAC ACCTCAAGTG		
15251		CTTATGAACA GAATACTTGT	 	
15301		CGGGGTTCTG GCCCCAAGAC		
15351		GACTGGGGTT CTGACCCCAA	 	
15401		AACGAAGCCT TTGCTTCGGA		
15451		CTTCACCCAC GAAGTGGGTG		
15501		CCTTCCAGGA GGAAGGTCCT		
15551	-	ATTCCCGCAC TAAGGGCGTG	•	
15601		CACCGAACAG GTGGCTTGTC	 	
15651		GCGCGGAAGA CGCGCCTTCT		
15701		GACATGAACG CTGTACTTGC		
15751		GGAGAAGCGC CCTCTTCGCG		
15801		CGCAACCCGA GCGTTGGGCT		
	CAAACCCCTG GTTTGGGGAC		 	
15901	ATGACAGCAC TACTGTCGTG			ATACAACTAC TATGTTGATG
15951	GGCGACCCTC CCGCTGGGAG	AGACCGGAAT TCTGGCCTTA		
160,01	CGTAACCTGC GCATTGGACG			GACATGATGC CTGTACTACG
16051	AAGACCCCGT TTCTGGGGCA			

Figure 26 Q

16101	GTGGGCGCCG CACCCGCGGC	A TGTTGCC T ACAACGG	CGTGCACTCC GCACGTGAGG	AAGAGCTTCT TTCTCGAAGA	ACAACGA CA TGTTGC ST
16151	GGCCGTCTAC	TCCCAACTCA	TCCGCCAGTT	TACCTCTCTG	ACCCACGTGT
	CCGGCAGATG	AGGGTTGAGT	AGGCGGTCAA	ATGGAGAGAC	TGGGTGCACA
16201	TCAATCGCTT	TCCCGAGAAC	CAGATTTTGG	CCCCCCCCC	AGCCCCCACC
	AGTTAGCGAA				
16251	ATCACCACCG	TCAGTGAAAA	CGTTCCTGCT	CTCACAGATC	ACGGGACGCT
				GACTGTCTAG	
16301	ACCGCTGCGC	AACAGCATCG	GAGGAGTCCA	GCGAGTGACC	ATTACTGACG
				CGCTCACTGG	
16351	CCAGACGCCG	CACCTGCCCC	TACGTTTACA	AGGCCCTGGG	CATAGTCTCG
				TCCGGGACCC	
16401	CCGCGCGTCC	TATCGAGCCG	CACTTTTTGA	GCAAGCATGT	CCATCCTTAT
				CGTTCGTACA	
16451	ATCGCCCAGC	AATAACACAG	GCTGGGGCCT	GCGCTTCCCA	AGCAAGATGT
	•			CGCGAAGGGT	
16501	TTGGCGGGGC	CAAGAAGCGC	TCCGACCAAC	ACCCAGTGCG	CGTGCGCGG
				TGGGTCACGC	
16551	CACTACCGCG	CGCCCTGGGG	CGCGCACAAA	CGCGGCCGCA	CTGGGCGCAC
				GCGCCGGCGT	
16601	CACCGTCGAT	GACGCCATCG	ACGCGGTGGT	GGAGGAGGCG	CGCAACTACA
				CCTCCTCCGC	
16651	CGCCCACGCC	GCCACCAGTG	TCCACAGTGG	ACGCGGCCAT TGCGCCGGTA	ACTOTOCO AC
				•	
16701	GTGCGCGGAG	CCCGGCGCTA	TGCTAAAATG	AAGAGACGGC TTCTCTGCCG	CCTCCCCCC
	CACGCGCCTC	GGGCCGCGAT	ACGATTTTAC	TICICIGCEG	CCICCGCGCA
16751	AGCACGTCGC	CACCGCCGCC	GACCCGGCAC	TGCCGCCCAA	CGCGCGGCGG
				ACGGCGGGTT	
16801	CGGCCCTGCT	TAACCGCGCA	CGTCGCACCG	GCCGACGGGC	GGCCATGCGG
				CGGCTGCCCG	
16851	GCCGCTCGAA	GGCTGGCCGC	GGGTATTGTC	ACTGTGCCCC	CCAGGTCCAG
					GGTCCAGGTC
16901	GCGACGAGCG	GCCGCCGCAG	CAGCCGCGGC	CATTAGTGCT	ATGACTCAGG
			•		TACTGAGTCC
16951	GTCGCAGGGG	CAACGTGTAT	TEGETECECE	ACTCGGTTAG	CGGCCTGCGC
					GCCGGACGCG
17001	CTCCCCGTGC	GCACCCGCCC	CCCGCGCAAC	TAGATTGCAA	GAAAAAACTA
	CACGGGCACG	CGTGGGCGGG	GGGCGCGTTG	ATCTAACGTT	CTTTTTTGAT



17051			TGTATCCAGC ACATAGGTCG		
17101			AAAGAAGAGA TTTCTTCTCT		
17151			GAAGGAAGAG CTTCCTTCTC		
17201		•••	AAAAGAAAGA TTTTCTTTCT		
17251			GCTACCGCGC CGATGGCGCG		
17301			TGTTTTGCGA ACAAAACGCT		
17351			CCCGCACCTA GGGCGTGGAT		
17401			CTTGAGCAGG GAACTCGTCC		
17451			TAAGGACATG ATTCCTGTAC		
17501			TAAAGCCCGT ATTTCGGGCA		
17551			GAAAAGCGCG CTTTTCGCGC		
17601			GCTGATGGTA CGACTACCAT		
17651			CCGTGGAACC GGCACCTTGG		
17701			GTGGCGCCGG CACCGCGGCC		
17751			CAGTAGCACC GTCATCGTGG		CCGCCACAGA GGCGGTGTCT
17801	GGGCATGGAG CCCGTACCTC	ACACAAACGT TGTGTTTGCA	CCCCGGTTGC GGGGCCAACG	CTCAGCGGTG GAGTCGCCAC	GCGGATGCCG CGCCTACGGC
17851	CGGTGCAGGC GCCACGTCCG	GGTCGCTGCG CCAGCGACGC	GCCGCGTCCA CGGCGCAGGT	AGACCTCTAC TCTGGAGATG	GGAGGTGCAA CCTCCACGTT
17901	ACGGACCCGT TGCCTGGGCA	GGATGTTTCG CCTACAAAGC	CGTTTCAGCC GCAAAGTCGG	CCCCGGCGCC	CGCGCCGTTC GCGCGGCAAG
17951	GAGGAAGTAC CTCCTTCATG				GCCCTACATC CGGGATGTAG

Figure 265

18001	CTTCCATTGC	GCCTACCCCC	GGCTATCGTG	GCTACACCTA!	CCCCCC MEA
		•		CGATGTGGAT	
18051	AGACGAGCAA	CTACCCGACG	CCGAACCACC	ACTGGAACCC	GCCGCCGCCG
	TCTGCTCGTT	GATGGGCTGC	GGCTTGGTGG	TGACCTTGGG	CGGCGGCGGC
18101	TCGCCGTCGC	CAGCCCGTGC	TGGCCCCGAT	TTCCGTGCGC	AGGGTGGCTC
	AGCGGCAGCG	GTCGGGCACG	ACCGGGGCTA	AAGGCACGCG	TCCCACCGAG
			•		
18151	GCGAAGGAGG	CAGGACCCTG	GTGCTGCCAA	CAGCGCGCTA	CCACCCCAGC
20202	CGCTTCCTCC	GTCCTGGGAC	CACGACGGTT	GTCGCGCGAT	GGTGGGGTCG
18201	ATCGTTTAAA	AGCCGGTCTT	TGTGGTTCTT	GCAGATATGG	CCCTCACCTG
••••	TAGCAAATTT	TCGGCCAGAA	ACACCAAGAA	CGTCTATACC	GGGAGTGGAC
	00000000000000000000000000000000000000	mmeeccorrec	CGGGATTCCG	AGGAAGAATG	CACCGTAGGA
18251	CCCCCCCCC	A A CCCCGG I GC	GCCCTAAGGC	TCCTTCTTAC	GTGGCATCCT
18301	GGGGCATGGC	CGGCCACGGC	CTGACGGGCG	GCATGCGTCG	TGCGCACCAC
				CGTACGCAGC	
18351	CGGCGGCGGC	GCGCGTCGCA	CCGTCGCATG	CGCGGCGGTA	TCCTGCCCCT
	GCCGCCGCCG	CGCGCAGCGT	GGCAGCGTAC	GCGCCGCCAT	AGGACGGGGA
18401	ССФФЪФФССЪ	CTGATCGCCG	CGGCGATTGG	CGCCGTGCCC	GGAATTGCAT
10401	GGAATAAGGT	GACTAGCGGC	GCCGCTAACC	GCGGCACGGG	CCTTAACGTA
		•			
18451	CCGTGGCCTT	GCAGGCGCAG	AGACACTGAT	TAAAAACAAG	TTGCATGTGG
	GGCACCGGAA	CGTCCGCGTC	TCTGTGACTA	ATTTTTGTTC	AACGTACACC
18501	AAAAATCAAA	ATAAAAAGTC	TGGACTCTCA	CGCTCGCTTG	GTCCTGTAAC
	TTTTTAGTTT	TATTTTTCAG	ACCTGAGAGT	GCGAGCGAAC	CAGGACATTG
18551	TATTITGTAG	AATGGAAGAC	ATCAACTTTG	CGTCTCTGGC	CCCGCGACAC
10331	ATAAAACATC	TTACCTTCTG	TAGTTGAAAC	GCAGAGACCG	GGGCGCTGTG
18601	CCCTCCCCC	CGTTCATGGG	AAACTGGCAA	GATATCGGCA	CCAGCAATAT
10001	CCGAGCGCGG	GCAAGTACCC	TTTGACCGTT	CTATAGCCGT	GGTCGTTATA
18651	GAGCGGTGGC	GCCTTCAGCT	GGGGCTCGCT	GTGGAGCGGC	TTAAAAATT
	CTCGCCACCG	CGGAAGTCGA	CCCCGAGCGA	CACCTCGCCG	TAATTTTTAA
18701	TCGGTTCCAC	CGTTAAGAAC	TATGGCAGGA	AGGCCTGGAA	CAGCAGCACA
••••	AGCCAAGGTG	GCAATTCTTG	ATACCGTCGT	TCCGGACCTT	GTCGTCGTGT
19751	GGCCAGATGC	TGAGGGATAA	GTTGAAAGAG	CAAAATTTCC	AACAAAAGGT
10/31	CCGGTCTACG	ACTCCCTATT	CAACTTTCTC	GTTTTAAAGG	TTGTTTTCCA
18801	GGTAGATGGC	CTGGCCTCTG	GCATTAGCGG	GCTGGTGGAC	CTGGCCAACC
	CCATCTACCG	GACCGGAGAC	CGTAATCGCC	CCACCACCTG	GACCGGTTGG
10051	AGGCAGTGCA	<u>አአ</u> ልጥ <u>አ</u> ልርልጥፕ	AACAGTAAGO	TTGATCCCCG	CCCTCCCGTA
10001	TCCGTCACCT	TITATTCTAA	TTGTCATTCG	AACTAGGGGC	GGGAGGGCAT
18901	GAGGAGCCTC	CACCGGCCGT	GGAGACAGTG	TCTCCAGAGG	GGCGTGGCGA
	CTCCTCGGAG	GTGGCCGGCA	CCTCTGTCAC	AGAGGTCTCC	CCGCACCGCT

Figure 26T

18951	AAAGCGTCCG TTTCGCAGGC	CCGACA GCGGGCTGT	GGGAAGAAAC CCCTTCTTTG	TCTGGTGACG AGACCACTGC	CAAATA G
19001	AGCCTCCCTC	GTACGAGGAG	GCACTAAAGC	AAGGCCTGCC	CACCACCCGT
	TCGGAGGGAG	CATGCTCCTC	CGTGATTTCG	TTCCGGACGG	CTGGTGGGCA
19051	CCCATCGCGC	CCATGGCTAC	CGGAGTGCTG	GGCCAGCACA	CACCCGTAAC
	GGGTAGCGCG	GGTACCGATG	GCCTCACGAC	CCGGTCGTGT	GTGGGCATTG
19101	GCTGGACCTG	CCTCCCCCG	CCGACACCCA	GCAGAAACCT	GTGCTGCCAG
	CGACCTGGAC	GGAGGGGGCC	GGCTGTGGGT	CGTCTTTGGA	CACGACGGTC
19151	GCCCGACCGC	CGTTGTTGTA	ACCCGTCCTA	GCCGCGCGTC	CCTGCGCCGC
	CGGGCTGGCG	GCAACAACAT	TGGGCAGGAT	CGGCGCGCAG	GGACGCGGCG
19201	GCCGCCAGCG	GTCCGCGATC	GTTGCGGCCC	GTAGCCAGTG	GCAACTGGCA
	CGGCGGTCGC	CAGGCGCTAG	CAACGCCGGG	CATCGGTCAC	CGTTGACCGT
19251	AAGCACACTG	AACAGCATCG	TGGGTCTGGG	GGTGCAATCC	CTGAAGCGCC
	TTCGTGTGAC	TTGTCGTAGC	ACCCAGACCC	CCACGTTAGG	GACTTCGCGG
19301	GACGATGCTT	CTGATAGCTA	ACGTGTCGTA	TGTGTGTCAT	GTATGCGTCC
	CTGCTACGAA	GACTATCGAT	TGCACAGCAT	ACACACAGTA	CATACGCAGG
19351			GCTGAGCCGC CGACTCGGCG		
19401	TGGCTACCCC	TTCGATGATG	CCGCAGTGGT	CTTACATGCA	CATCTCGGGC
	ACCGATGGGG	AAGCTACTAC	GGCGTCACCA	GAATGTACGT	GTAGAGCCCG
19451	CAGGACGCCT	CGGAGTACCT	GAGCCCCGGG	CTGGTGCAGT	TTGCCCGCGC
	GTCCTGCGGA	GCCTCATGGA	CTCGGGGCCC	GACCACGTCA	AACGGGCGCG
19501	CACCGAGACG GTGGCTCTGC	TACTTCAGCC ATGAAGTCGG	TGAATAACAA ACTTATTGTT	GTTTAGAAAC CAAATCTTTG	CCCACGGTGG
19551	CGCCTACGCA	CGACGTGACC	ACAGACCGGT	CCCAGCGTTT	GACGCTGCGG
	GCGGATGCGT	GCTGCACTGG	TGTCTGGCCA	GGGTCGCAAA	CTGCGACGCC
19601	TTCATCCCTG	TGGACCGTGA	GGATACTGCG	TACTCGTACA	AGGCGCGGTT
	AAGTAGGGAC	ACCTGGCACT	CCTATGACGC	ATGAGCATGT	TCCGCGCCAA
19651	CACCCTAGCT	GTGGGTGATA	ACCGTGTGCT	GGACATGGCT	TCCACGTACT
	GTGGGATCGA	CACCCACTAT	TGGCACACGA	CCTGTACCGA	AGGTGCATGA
19701	TTGACATCCG AACTGTAGGC	CGGCGCACGAC	GACAGGGGCC CTGTCCCCGG	CTACTTTTAA GATGAAAATT	GCCCTACTCT
19751	GGCACTGCCT	ACAACGCCCT	GGCTCCCAAG	GGTGCCCCAA	ATCCTTGCGA
	CCGTGACGGA	TGTTGCGGGA	CCGAGGGTTC	CCACGGGGTT	TAGGAACGCT
19801	ATGGGATGAA	GCTGCTACTG	CTCTTGAAAT	AAACCTAGAA	GAAGAGGACG
	TACCCTACTT	CGACGATGAC	GAGAACTTTA	TTTGGATCTT	CTTCTCCTGC
19851	ATGACAACGA	AGACGAAGTA	GACGAGCAAG	CTGAGCAGCA	AAAAACTCAC
	TACTGTTGCT	TCTGCTTCAT	CTGCTCGTTC	GACTCGTCGT	TTTTTGAGTG

Figure 26 U

19901	GTATTTGGGC CATAAACCCG	A CCCCTTA TCCGCGGAAT	TTCTGGTATA AAGACCATAT	AATATTACAA TTATAATGTT	AGGAGG T TCCTCCCATA
19951	TCAAATAGGT AGTTTATCCA	GTCGAAGGTC CAGCTTCCAG	AAACACCTAA TTTGTGGATT	ATATGCCGAT TATACGGCTA	AAAACATTTC TTTTGTAAAG
20001	AACCTGAACC TTGGACTTGG	TCAAATAGGA AGTTTATCCT	GAATCTCAGT CTTAGAGTCA	GGTACGAAAC CCATGCTTTG	AGAAATTAAT TCTTTAATTA
20051	GTACGTCGAC	GGAGAGTCCT CCTCTCAGGA	TTTTTTCTGA	TGGGGTTACT	TTGGTACAAT
20101	GCCAAGTATA	GCAAAACCCA CGTTTTGGGT	GTTTACTTTT	ACCTCCCGTT	CCGTAAGAAC
20151	ATTTCGTTGT	AAATGGAAAG TTTACCTTTC	GATCTTTCAG	TTCACCTTTA	CGTTAAAAAG
20201	-	TCCGTCGGCG	TCCGTTACCA	CTATTGAACT	GAGGATTTCA
20251	CCATAACATG	AGTGAAGATG TCACTTCTAC	ATCTATATCT	TTGGGGTCTG	TGAGTATAAA
20301	GAATGTACGG	CACTATTAAG GTGATAATTC	CTTCCATTGA	GTGCTCTTGA	TTACCCGGTT
20351	GTTAGATACG	CCAACAGGCC GGTTGTCCGG	ATTAATGTAA	CGAAAATCCC	TGTTAAAATA
20401	ACCAGATTAC	TATTACAACA ATAATGTTGT	CGTGCCCATT	ATACCCACAA	GACCGCCCGG
20451	TTCGTAGCGT	GTTGAATGCT CAACTTACGA	CAACATCTAA	ACGTTCTGTC	TTTGTGTCTC
20501	CTTTCATACC GAAAGTATGG	AGCTTTTGCT TCGAAAACGA	TGATTCCATT ACTAAGGTAA	GGTGATAGAA CCACTATCTT	CCAGGTACTT GGTCCATGAA
20551	AAGATACACC	AATCAGGCTG TTAGTCCGAC	AACTGTCGAT	ACTAGGTCTA	CAATCTTAAT
20601	AACTTTTAGT	TGGAACTGAA ACCTTGACTT	CTACTTGAAG	GTTTAATGAC	GAAAGGTGAC
		AATTATGTCT	CTGAGAATGG	TTCCATTTTG	GATTTTGTCC
		CCTACCCTTT	TTCTACGATG	TCTTAAAAGT	CTATTTTTAC
20751	AAATAAGAGT TTTATTCTCA	TGGAAATAAT ACCTTTATTA	TTTGCCATGG AAACGGTACC	AAATCAATCT TTTAGTTAGA	AAATGCCAAC TTTACGGTTG
20801	CTGTGGAGAA GACACCTCTT	ATTTCCTGTA TAAAGGACAT	CTCCAACATA GAGGTTGTAT	GCGCTGTATT CGCGACATAA	TGCCCGACAA ACGGGCTGTT

Figure 26 V

20851	GCTAAAGTAC	ACCTTCCA	ACGTAAAAAT	TTCTGATÄÄČ	TCARACIOTT
	CGATTTCATG	T GGAAGGT	TGCATTTTTA	AAGACTATTG	GCTTTC A
20901	ACGACTACAT	GAACAAGCGA	CTGGTGGCTC	CCGGGCTAGT	GGACTGCTAC
	TGCTGATGTA	CITGTTCGCT	CACCACCGAG	GGCCCGATCA	CCTGACGATG
20951	ATTAACCTTG	GAGCACGCTG	GTCCCTTGAC	TATATGGACA	ACGTCAACCC
	TAATTGGAAC	CTCGTGCGAC	CAGGGAACTG	ATATACCTGT	TGCAGTTGGG
21001	ATTTAACCAC	CACCGCAATG	CTGGCCTGCG	CTACCGCTCA	ATGTTGCTGG
	TAAATTGGTG	GTGGCGTTAC	GACCGGACGC	GATGGCGAGT	TACAACGACC
21051	GCAATGGTCG	CTATGTGCCC	TTCCACATCC	AGGTGCCTCA	GAAGTTCTTT
	CGTTACCAGC	GATACACGGG	AAGGTGTAGG	TCCACGGAGT	CTTCAAGAAA
21101	GCCATTAAAA CGGTAATTTT	ACCTCCTTCT TGGAGGAAGA	CCTGCCGGGC	TCATACACCT AGTATGTGGA	ACGAGTGGAA TGCTCACCTT
21151	CTTCAGGAAG	GATGTTAACA	TGGTTCTGCA	GAGCTCCCTA	GGAAATGACC
	GAAGTCCTTC	CTACAATTGT	ACCAAGACGT	CTCGAGGGAT	CCTTTACTGG
21201	TAAGGGTTGA	CGGAGCCAGC	ATTAAGTTTG	ATAGCATTTG	CCTTTACGCC
	ATTCCCAACT	GCCTCGGTCG	TAATTCAAAC	TATCGTAAAC	GGAAATGCGG
21251	ACCTTCTTCC	CCATGGCCCA	CAACACCGCC	TCCACGCTTG	AGGCCATGCT
	TGGAAGAAGG	GGTACCGGGT	GTTGTGGCGG	AGGTGCGAAC	TCCGGTACGA
21301	TAGAAACGAC	ACCAACGACC	AGTCCTTTAA	CGACTATCTC	TCCGCCGCCA
	ATCTTTGCTG	TGGTTGCTGG	TCAGGAAATT	GCTGATAGAG	AGGCGGCGGT
21351	ACATGCTCTA	CCCTATACCC	GCCAACGCTA	CCAACGTGCC	CATATCCATC
	TGTACGAGAT	GGGATATGGG	CGGTTGCGAT	GGTTGCACGG	GTATAGGTAG
21401	CCCTCCCGCA	ACTGGGCGGC	TTTCCGCGGC	TGGGCCTTCA	CGCGCCTTAA
	GGGAGGGCGT	TGACCCGCCG	AAAGGCGCCG	ACCCGGAAGT	GCGCGGAATT
21451	GACTAAGGAA	ACCCCATCAC	TGGGCTCGGG	CTACGACCCT	TATTACACCT
	CTGATTCCTT	TGGGGTAGTG	ACCCGAGCCC	GATGCTGGGA	ATAATGTGGA
21501	ACTCTGGCTC	TATACCCTAC	CTAGATGGAA	CCTTTTACCT	CAACCACACC
	TGAGACCGAG	ATATGGGATG	GATCTACCTT	GGAAAATGGA	GTTGGTGTGG
21551	TTTAAGAAGG AAATTCTTCC	TGGCCATTAC ACCGGTAATG	CTTTGACTCT GAAACTGAGA	TCTGTCAGCT AGACAGTCGA	GGCCTGGCAA
21601	TGACCGCCTG	CTTACCCCCA	ACGAGTITGA	AATTAAGCGC	TCAGTTGACG
	ACTGGCGGAC	GAATGGGGGT	TGCTCAAACI	TTAATTCGCG	AGTCAACTGC
21651	GGGAGGGTTA	CAACGTTGCC	CAGTGTAACA	TGACCAAAGA	CTGGTTCCTG
	CCCTCCCAAT	GTTGCAACGG	GTCACATTGT	ACTGGTTTCT	GACCAAGGAC
21701	GTACAAATGC CATGTTTACG	TAGCTAACTA ATCGATTGAT	TAACATTGGC	TACCAGGGCT ATGGTCCCGA	TCTATATCCC AGATATAGGG
21751	AGAGAGCTAC TCTCTCGATG	AAGGACCGCA TTCCTGGCGI	TGTACTCCTT ACATGAGGAF	CTTTAGAAAC A GAAATCTTTG	TTCCAGCCCA AAGGTCGGGT

Figure 26 W

21801	TGAGCCGTCA ACTCGGCAGT	GETGGTGGAT CACCTA	GATACŤAAĄT CTATGATTTA	ACAAGGACTA <sup>©</sup> TGTTCCTGAT	ECAACARTICO GCTTGT CC
21851	GGCATCCTAC	ACCAACACAA	CAACTCTGGA	TTTGTTGGCT	ACCTTGCCCC
				AAACAACCGA	
21901	CACCATGCGC	GAAGGACAGG	CCTACCCTGC	TAACTTCCCC	TATCCGCTTA
	GTGGTACGCG	CTTCCTGTCC	GGATGGGACG	ATTGAAGGGG	ATAGGCGAAT
21951	TAGGCAAGAC	CGCAGTTGAC	ACCATTACCC	AGAAAAAGTT	TCTTTGCGAT
				TCTTTTTCAA	
22001	CGCACCCTTT	GGCGCATCCC	ATTCTCCAGT	AACTTTATGT	CCATGGGCGC
	GCGTGGGAAA	CCGCGTAGGG	TAAGAGGTCA	TTGAAATACA	GGTACCCGCG
22051	ACTCACAGAC	CTGGGCCAAA	ACCTTCTCTA	CGCCAACTCC	GCCCACGCGC
	TGAGTGTCTG	GACCCGGTTT	TGGAAGAGAT	GCGGTTGAGG	CGGGTGCGCG
22101	TAGACATGAC	TTTTGAGGTG	GATCCCATGG	ACGAGCCCAC	CCTTCTTTAT
	ATCTGTACTG	AAAACTCCAC	CTAGGGTACC	TGCTCGGGTG	GGAAGAAATA
22151	GTTTTGTTTG	AAGTCTTTGA	CGTGGTCCGT	GTGCACCAGC	CGCACCGCGG
	CAAAACAAAC	TTCAGAAACT	GCACCAGGCA	CACGTGGTCG	GCGTGGCGCC
22201	CGTCATCGAA	ACCGTGTACC	TGCGCACGCC	CTTCTCGGCC	GGCAACGCCA
	GCAGTAGCTT	TGGCACATGG	ACGCGTGCGG	GAAGAGCCGG	CCGTTGCGGT
22251	CAACATAAAG	AAGCAAGCAA	CATCAACAAC	AGCTGCCGCC	ATGGGCTCCA
	GTTGTATTTC	TTCGTTCGTT	GTAGTTGTTG	TCGACGGCGG	TACCCGAGGT
22301	GTGAGCAGGA	ACTGAAAGCC	ATTGTCAAAG	ATCTTGGTTG	TGGGCCATAT
	CACTCGTCCT	TGACTTTCGG	TAACAGTTTC	TAGAACCAAC	ACCCGGTATA
22351	TTTTTGGGCA	CCTATGACAA	GCGCTTTCCA	GGCTTTGTTT	CTCCACACAA
	AAAAACCCGT	GGATACTGTT	CGCGAAAGGT	CCGAAACAAA	GAGGTGTGTT
22401	GCTCGCCTGC	GCCATAGTCA	ATACGGCCGG	TCGCGAGACT	GGGGGCGTAC
	CGAGCGGACG	CGGTATCAGT	TATGCCGGCC	AGCGCTCTGA	CCCCCGCATG
22451	ACTGGATGGC	CTTTGCCTGG	AACCCGCACT	CAAAAACATG	CTACCTCTTT
	TGACCTACCG	GAAACGGACC	TTGGGCGTGA	GTTTTTGTAC	GATGGAGAAA
22501	GAGCCCTTTG	GCTTTTCTGA	CCAGCGACTC	AAGCAGGTTT	ACCAGTTTGA
	CTCGGGAAAC	CGAAAAGACT	GGTCGCTGAG	TTCGTCCAAA	TGGTCAAACT
22551	GTACGAGTCA	CTCCTGCGCC	GTAGCGCCAT	TGCTTCTTCC	CCCGACCGCT
	CATGCTCAGT	GAGGACGCGG	CATCGCGGTA	ACGAAGAAGG	GGGCTGGCGA
22601	GTATAACGCT	GGAAAAGTCC	ACCCAAAGCG	TACAGGGGCC	CAACTCGGCC
	CATATTGCGA	CCTTTTCAGG	TGGGTTTCGC	ATGTCCCCGG	GTTGAGCCGG
22651	GCCTGTGGAC	TATTCTGCTG	CATGTTTCTC	CACGCCTTTG	CCAACTGGCC
	CGGACACCTG	ATAAGACGAC	GTACAAAGAG	GTGCGGAAAC	GGTTGACCGG
22701	CCAAACTCCC	ATGGATCACA	ACCCCACCAT	GAACCTTATT	ACCGGGGTAC
	GGTTTGAGGG	TACCTAGTGT	TGGGGTGGTA	CTTGGAATAA	TGGCCCCATG

Figure 26 X

22751				ACCCCACGEN CGCACCONSC:
22801	CAGGAACAGC	TCTACAGCTT	CCTGGAGCGC	CACTCGCCCT ACTTCCGCAG
	GTCCTTGTCG	AGATGTCGAA	GGACCTCGCG	GTGAGCGGGA TGAAGGCGTC
22851	CCACAGTGCG	CAGATTAGGA	GCGCCACTTC	TTTTTGTCAC TTGAAAAACA AAAAACAGTG AACTTTTTGT
22901	TGTAAAAATA	ATGTACTAGA	GACACTTTCA	ATAAAGGCAA ATGCTTTTAT
	ACATTTTTAT	TACATGATCT	CTGTGAAAGT	TATTTCCGTT TACGAAAATA
22951	TTGTACACTC	TCGGGTGATT	ATTTACCCCC	ACCCTTGCCG TCTGCGCCGT
	AACATGTGAG	AGCCCACTAA	TAAATGGGGG	TGGGAACGGC AGACGCGGCA
23001	TTAAAAATCA	AAGGGGTTCT	GCCGCGCATC	GCTATGCGCC ACTGGCAGGG
	AATTTTTAGT	TTCCCCAAGA	CGGCGCGTAG	CGATACGCGG TGACCGTCCC
23051	ACACGTTGCG	ATACTGGTGT	TTAGTGCTCC	ACTTAAACTC AGGCACAACC
	TGTGCAACGC	TATGACCACA	AATCACGAGG	TGAATTTGAG TCCGTGTTGG
23101	ATCCGCGGCA	GCTCGGTGAA	GTTTTCACTC	CACAGGCTGC GCACCATCAC
	TAGGCGCCGT	CGAGCCACTT	CAAAAGTGAG	GTGTCCGACG CGTGGTAGTG
23151	CAACGCGTTT	AGCAGGTCGG	GCGCCGATAT	CTTGAAGTCG CAGTTGGGGC
	GTTGCGCAAA	TCGTCCAGCC	CGCGGCTATA	GAACTTCAGC GTCAACCCCG
23201	CTCCGCCCTG GAGGCGGGAC	CGCGCGCGAG GCGCGCGCTC	TTGCGATACA AACGCTATGT	CAGGGTTGCA GCACTGGAAC GTCCCAACGT CGTGACCTTG
23251	ACTATCAGCG	CCGGGTGGTG	CACGCTGGCC	AGCACGCTCT TGTCGGAGAT
	TGATAGTCGC	GGCCCACCAC	GTGCGACCGG	TCGTGCGAGA ACAGCCTCTA
23301	CAGATCCGCG GTCTAGGCGC	TCCAGGTCCT AGGTCCAGGA	CCGCGTTGCT	CAGGGCGAAC GGAGTCAACT GTCCCGCTTG CCTCAGTTGA
23351	TTGGTAGCTG	CCTTCCCAAA	AAGGGCGCGT	GCCCAGGCTT TGAGTTGCAC
	AACCATCGAC	GGAAGGGTTT	TTCCCGCGCA	CGGGTCCGAA ACTCAACGTG
23401	TCGCACCGTA AGCGTGGCAT	GTGGCATCAA CACCGTAGTT	AAGGTGACCG	TGCCCGGTCT GGGCGTTAGG ACGGGCCAGA CCCGCAATCC
23451	ATACAGCGCC	TGCATAAAAG	CCTTGATCTO	CTTAAAAGCC ACCTGAGCCT
	TATGTCGCGG	ACGTATTTTC	GGAACTAGAC	CGAATTTTCGG TGGACTCGGA
23501	TTGCGCCTTC	AGAGAAGAAC	ATGCCGCAAG	ACTTGCCGGA AAACTGATTG
	AACGCGGAAG	TCTCTTCTTC	TACGGCGTTC	TGAACGGCCT TTTGACTAAC
23551	GCCGGACAGG CGGCCTGTCC	CCGCGTCGTC	CACGCAGCAC CGTGCGTCGTC	CTTGCGTCGG TGTTGGAGAT GAACGCAGCC ACAACCTCTA
23601	CTGCACCACA GACGTGGTGT	TTTCGGCCCC	ACCGGTTCT	CACGATCTTG GCCTTGCTAG  GTGCTAGAAC CGGAACGATC
23651	ACTGCTCCTT TGACGAGGAA	CAGCGCGCGC	TGCCCGTTT	COCTCGTCAC ATCCATTTCA A GCGAGCAGTG TAGGTAAAGT

France 26 Y

23701	ATCACGTGCT TAGTGCACGA	CC TATTTAT GGAATAAATA	CATAATGCTT GTATTACGAA	CCGTGTAGAC GGCACATCTG	ACTTAA TC TGAATTCGAG
23751	GCCTTCGATC CGGAAGCTAG	TCAGCGCAGC AGTCGCGTCG	GGTGCAGCCA CCACGTCGGT	CAACGCGCAG GTTGCGCGTC	CCCGTGGGCT GGGCACCCGA
23801	CGTGATGCTT GCACTACGAA	GTAGGTCACC CATCCAGTGG	TCTGCAAACG AGACGTTTGC	ACTGCAGGTA TGACGTCCAT	CGCCTGCAGG GCGGACGTCC
23851	AATCGCCCCA TTAGCGGGGT	TCATCGTCAC AGTAGCAGTG	AAAGGTCTTG TTTCCAGAAC	TTGCTGGTGA AACGACCACT	AGGTCAGCTG TCCAGTCGAC
23901	_	ACGAGGAGCA	ACTCGCTCCA	GAACGTATGC	CGGCGGTCTC
23951	GAAGGTGAAC	GTCAGGCAGT CAGTCCGTCA	TCAAACTTCA	AGCGGAAATC	TAGCAATAGG
24001	TGCACCATGA	TGTCCATCAG ACAGGTAGTC	GCGCGCGCGT	CGGAGGTACG	GGAAGAGGGT
24051	CGCAGACACG GCGTCTGTGC	ATCGGCACAC TAGCCGTGTG	TCAGCGGGTT AGTCGCCCAA	CATCACCGTA GTAGTGGCAT	ATTTCACTTT TAAAGTGAAA
24101	CCGCTTCGCT GGCGAAGCGA	GGGCTCTTCC CCCGAGAAGG	TCTTCCTCTT AGAAGGAGAA	GCGTCCGCAT CGCAGGCGTA	ACCACGCGCC TGGTGCGCGG
24151	ACTGGGTCGT TGACCCAGCA	CTTCATTCAG GAAGTAAGTC	CCGCCGCACT	GTGCGCTTAC CACGCGAATG	CTCCTTTGCC GAGGAAACGG
24201	TACGAACTAA	TCGTGGCCAC	CCAACGACTT	TGGGTGGTAA	
24251	GTAGAAGAGA	TTCTTCCTCG AAGAAGGAGC	GACAGGTGCT	AATGGAGACC	ACTACCGCCC
24301	GCGAGCCCGA	TGGGAGAAGG ACCCTCTTCC	CGCGAAGAAA	AAGAAGAACC	CGCGTTACCG
24351	CAAATCCGCC GTTTAGGCGG	GCCGAGGTCG CGGCTCCAGC	ATGGCCGCGG TACCGGCGCC	GCTGGGTGTG CGACCCACAC	CGCGGCACCA
24401	CGCGCAGAAC	ACTACTCAGA	AGGAGCAGGA	GCCTGAGCTA	ACGCCGCCTC TGCGGCGGAG
	TAGGCGAAAA	AACCCCCGCG	GGCCCCTCCG	CCGCCGCTGC	GGGACGGGGA CCCTGCCCCT
	GCTGTGCAGG	AGGTACCAAC	CCCCTGCAGC	GCGGCGTGGC	CGTCCGCGCT GCAGGCGCGA
24551	CGGGGGTGGT GCCCCCACCA	TTCGCGCTGC AAGCGCGACG	TCCTCTTCCC AGGAGAAGGG	GACTGGCCAT CTGACCGGTA	TTCCTTCTCC AAGGAAGAGG
24601	TATAGGCAGA ATATCCGTCT	AAAAGATCAT TTTTCTAGTA	GGAGTCAGTC CCTCAGTCAG	GAGAAGAAGG CTCTTCTTCC	ACAGCCTAAC TGTCGGATTG

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24651	CGCCCCCTCT GCGGGGGAGA	QTTCGCCA CTCAAGCGGT	CCACCGCCTC GGTGGCGGAG	CACCGATGCC CTGGCTACGG	GUCAAC CGGTTGC-CG
24701	CTACCACCTT	CCCCGTCGAG	GCACCCCCGC	TTGAGGAGGA	GGAAGTGATT
	GATGGTGGAA	GGGGCAGCTC	CGTGGGGGCG	AACTCCTCCT	CCTTCACTAA
24751	ATCGAGCAGG	ACCCAGGTTT	TGTAAGCGAA	GACGACGAGG	ACCGCTCAGT
	TAGCTCGTCC	TGGGTCCAAA	ACATTCGCTT	CTGCTGCTCC	TGGCGAGTCA
24801	ACCAACAGAG	GATAAAAAGC	AAGACCAGGA	CAACGCAGAG	GCAAACGAGG
	TGGTTGTCTC	CTATTTTTCG	TTCTGGTCCT	GTTGCGTCTC	CGTTTGCTCC
24851	AACAAGTCGG	GCGGGGGGAC	GAAAGGCATG	GCGACTACCT	AGATGTGGGA
	TTGTTCAGCC	CCCCCCTC	CTTTCCGTAC	CGCTGATGGA	TCTACACCCT
24901	GACGACGTGC	TGTTGAAGCA	TCTGCAGCGC	CAGTGCGCCA	TTATCTGCGA
			AGACGTCGCG		
24951	CGCGTTGCAA	GAGCGCAGCG	ATGTGCCCCT	CGCCATAGCG	GATGTCAGCC
			TACACGGGGA		
25001	TTGCCTACGA	ACGCCACCTA	TTCTCACCGC	GCGTACCCCC	CAAACGCCAA
			AAGAGTGGCG		
25051	GAAAACGGCA	CATGCGAGCC	CAACCCGCGC	CTCAACTTCT	ACCCCGTATT
ı			CTTCCCCCC		
25101	TGCCGTGCCA	GAGGTGCTTG	CCACCTATCA	CATCTTTTTC	CAAAACTGCA
			GGTGGATAGT		
25151	AGATACCCCT	ATCCTGCCGT	GCCAACCGCA CGGTTGGCGT	CCCCAGCGGA	CAAGCAGCIG
				•	
25201	GCCTTGCGGC	AGGGCGCTGT	CATACCTGAT GTATGGACTA	TARCECCI CGC	ACTITICATION A
	•				
25251	GCCAAAAATC	TTTGAGGGTC	TTGGACGCGA	CGAGAAGCGC	GCGGCAAACG
			AACCTGCGCT		
25301	CTCTGCAACA	GGAAAACAGC	GAAAATGAAA	GTCACTCTGG	AGTGTTGGTG
			CTTTTACTTT		
25351	GAACTCGAGG	GTGACAACGC	GCGCCTAGCC	GTACTAAAAC	GCAGCATCGA
			CGCGGATCGG		
25401	GGTCACCCAC	TTTGCCTACC	CGGCACTTAA	CCTACCCCC	AAGGTCATGA
					TTCCAGTACT
25451	GCACAGTCAT	GAGTGAGCTG	ATCGTGCGCC	GTGCGCAGCC	CCTGGAGAGG
					GGACCTCTCC
25501	GATGCAAATT	TGCAAGAACA	AACAGAGGAG	GCCTACCCG	CAGTTGGCGA
	CTACGTTTAA	ACGTTCTTGT	TTGTCTCCTC	: CCGGATGGGC	GTCAACCGCT
25551	CGAGCAGCTA	GCGCGCTGGC	TTCAAACGCG	CGAGCCTGC	GACTTGGAGG
	GCTCGTCGAT	CGCGCGACCG	AAGTTTGCGC	GCTCGGACG	CTGAACCTCC

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25601	TCGCTGCGTT	AATGATG TGATTACTAC	CGGCGTCACG	AGCAATGGCA	CCTCGAACTC
25651	TGCATGCAGC	GGTTCTTTGC	TGACCCGGAG	ATGCAGCGCA	AGCTAGAGGA
	ACGTACGTCG	CCAAGAAACG	ACTGGGCCTC	TACGTCGCGT	TCGATCTCCT
25701	AACATTGCAC	TACACCTTTC	GACAGGGCTA	CGTACGCCAG	GCCTGCAAGA
	TTGTAACGTG	ATGTGGAAAG	CTGTCCCGAT	GCATGCGGTC	CGGACGTTCT
25751	TCTCCAACGT	GGAGCTCTGC	AACCTGGTCT	CCTACCTTGG	AATTTTGCAC
	AGAGGTTGCA	CCTCGAGACG	TTGGACCAGA	GGATGGAACC	TTAAAACGTG
25801	GAAAACCGCC	TTGGGCAAAA	CGTGCTTCAT	TCCACGCTCA	AGGGCGAGGC
	CTTTTGGCGG	AACCCGTTTT	GCACGAAGTA	AGGTGCGAGT	TCCCGCTCCG
25851	GCGCCGCGAC	TACGTCCGCG	ACTGCGTTTA	CTTATTTCTA	TGCTACACCT
	CGCGGCGCTG	ATGCAGGCGC	TGACGCAAAT	GAATAAAGAT	ACGATGTGGA
25901	GGCAGACGGC	CATGGGCGTT	TGGCAGCAGT	GCTTGGAGGA	GTGCAACCTC
	CCGTCTGCCG	GTACCCGCAA	ACCGTCGTCA	CGAACCTCCT	CACGTTGGAG
25951		AGAAACTGCT TCTTTGACGA			
26001	CTTCAACGAG	CGCTCCGTGG	CCGCGCACCT	GGCGGACATC	ATTTTCCCCG
	GAAGTTGCTC	GCGAGGCACC	GGCGCGTGGA	CCGCCTGTAG	TAAAAGGGGC
26051	AACGCCTGCT	TAAAACCCTG	CAACAGGGTC	TGCCAGACTT	CACCAGTCAA
	TTGCGGACGA	ATTTTGGGAC	GTTGTCCCAG	ACGGTCTGAA	GTGGTCAGTT
26101	AGCATGTTGC	AGAACTTTAG	GAACTTTATC	CTAGAGCGCT	CAGGAATCTT
	TCGTACAACG	TCTTGAAATC	CTTGAAATAG	GATCTCGCGA	GTCCTTAGAA
26151	GCCCGCCACC	TGCTGTGCAC	TTCCTAGCGA	CTTTGTGCCC	ATTAAGTACC
	CGGGCGGTGG	ACGACACGTG	AAGGATCGCT	GAAACACGGG	TAATTCATGG
26201	GCGAATGCCC	TCCGCCGCTT	TGGGGCCACT	GCTACCTTCT	GCAGCTAGCC
	CGCTTACGGG	AGGCGGCGAA	ACCCCGGTGA	CGATGGAAGA	CGTCGATCGG
26251	AACTACCTTG	CCTACCACTC	TGACATAATG	GAAGACGTGA	GCGGTGACGG
	TTGATGGAAC	GGATGGTGAG	ACTGTATTAC	CTTCTGCACT	CGCCACTGCC
26301	TCTACTGGAG AGATGACCTC	TGTCACTGTC ACAGTGACAG	GCTGCAACCT CGACGTTGGA	ATGCACCCCG TACGTGGGGC	CACCGCTCCC
26351	TGGTTTGCAA ACCAAACGTT	TTCGCAGCTG AAGCGTCGAC	CTTAACGAAA GAATTGCTTT	GTCAAATTAT CAGTTTAATA	CGGTACCTTT
26401	GAGCTGCAGG	GTCCCTCGCC	TGACGAAAAG	TCCGCGGCTC	CGGGGTTGAA
	CTCGACGTCC	CAGGGAGCGG	ACTGCTTTTC	AGGCGCCGAG	GCCCCAACTT
26451	ACTCACTCCG	GGGCTGTGGA	CGTCGGCTTA	CCTTCGCAAA	TTTGTACCTG
	TGAGTGAGGC	CCCGACACCT	GCAGCCGAAT	GGAAGCGTTT	AAACATGGAC
26501	AGGACTACCA	CGCCCACGAG	ATTAGGTTCT	ACGAAGACCA	ATCCCGCCCG
	TCCTGATGGT	GCGGGTGCTC	TAATCCAAGA	TGCTTCTGGT	TAGGGCGGGC

Figure 26 AB

26551		A TACCGC TCGAATGGCG			
26601		GCCATCAACA CGGTAGTTGT			
26651	GACGGGGGGT CTGCCCCCCA	TTACTTGGAC AATGAACCTG			
26701		CGCAGCCCTA GCGTCGGGAT			
26751	•	Caaaaagaag Gtttttcttc			
26801		GGGACAGTCA CCCTGTCAGT			
26851		GAAGACTGGG CTTCTGACCC			
26901		AGACGAAACA TCTGCTTTGT			
26951		AATCGGCAAC TTAGCCGTTG			
27001		CCGGCACTGC GGCCGTGACG			
27051		CAGGGCCGGT GTCCCGGCCA			
27101		AGCGCCAAGG TCGCGGTTCC			
27151					
		TGCTTGCAAG ACGAACGTTC			
27201	GTATCAACGA GCTTTCTTCT	ACGAACGTTC CTACCATCAC	TGACACCCCC GGCGTGGCCT	CTTGTAGAGG TCCCCCGTAA	AAGCGGGCGG
27201	GTATCAACGA GCTTTCTTCT CGAAAGAAGA TACTACCGTC	ACGAACGTTC CTACCATCAC GATGGTAGTG ATCTCTACAG	TGACACCCCC GGCGTGGCCT CCGCACCGGA CCCATACTGC	CTTGTAGAGG TCCCCCGTAA AGGGGGCATT ACCGGCGGCA	AAGCGGGCGG
27251	GTATCAACGA GCTTTCTTCT CGAAAGAAGA TACTACCGTC ATGATGGCAG CAGCAGCGGC	ACGAACGTTC CTACCATCAC GATGGTAGTG ATCTCTACAG TAGAGATGTC CACACAGAAG	TGACACCCCC  GGCGTGGCCT CCGCACCGGA  CCCATACTGC GGGTATGACG CAAAGGCGAC	CTTCTAGAGG TCCCCCGTAA AGGGGGCATT ACCGGCGCCA TGGCCGCCGT CGGATAGCAA	AAGCGGGCGG CATCCTGCAT GTAGGACGTA GCGGCAGCAA
27251 27301	GTATCAACGA GCTTTCTTCT CGAAAGAAGA TACTACCGTC ATGATGGCAG CAGCAGCGCGC GTCGTCGCCG AAGCCCAAGA	ACGAACGTTC CTACCATCAC GATGGTAGTG ATCTCTACAG TAGAGATGTC CACACAGAAG GTGTGTCTTC AATCCACAGC	TGACACCCCC GGCTGGCCT CCGCACCGGA CCCATACTGC GGGTATGACG CAAAGGCGAC GTTTCCGCTG GGCGGCAGCA	CTTGTAGAGG TCCCCGTAA AGGGGGCATT ACCGGCGGCA TGGCCGCCGT CGGATAGCAA GCCTATCGTT GCAGGAGGAG	AAGCGGGCGG CATCCTGCAT GTAGGACGTA GCGGCAGCAA CGCCGTCGTT GACTCTGACA CTGAGACTGT
27251 27301 27351	GTATCAACGA GCTTTCTTCT CGAAAGAAGA TACTACCGTC ATGATGGCAG CAGCAGCGCC GTCGTCGCCG AAGCCCAAGA TTCGGGTTCT TCTGGCGCCC	ACGAACGTTC CTACCATCAC GATGGTAGTG ATCTCTACAG TAGAGATGTC CACACAGAAG GTGTGTCTTC AATCCACAGC TTAGGTGTCG AACGAACCCG	TGACACCCCC GGCTGGCCT CCGCACCGGA CCCATACTGC GGGTATGACG CAAAGGCGAC GTTTCCGCTG GGCGGCAGCA CCGCCGTCGT TATCGACCCC	TCCCCGTAA AGGGGGCATT ACCGGCGGCA TGGCCGCCGT CGGATAGCAA GCCTATCGTT GCAGGAGGAG CGTCCTCCTC	AAGCGGGCGG CATCCTGCAT GTAGGACGTA GCGGCAGCAA CGCCGTCGTT GACTCTGACA CTGAGACTGT GAGCGCTGCG CTCGCGACGC

Figure 26 AC

27501	CTGAAAATAA GACTTTTATT	A CAGGTC TTTTGTCCAG	TCTGCGATCC AGACGCTAGG	CTCACCCGCA GAGTGGGCGT	GCTGCC A CGACGGACAT
27551	TCACAAAAGC AGTGTTTTCG	GAAGATCAGC CTTCTAGTCG	TTCGGCGCAC AAGCCGCGTG	GCTGGAAGAC CGACCTTCTG	GCGGAGGCTC CGCCTCCGAG
27601	TCTTCAGTAA AGAAGTCATT	ATACTGCGCG TATGACGCGC	CTGACTCTTA GACTGAGAAT	AGGACTAGTT TCCTGATCAA	TCGCGCCCTT AGCGCGGGAA
27651	TCTCAAATTT AGAGTTTAAA	AAGCGCGAAA TTCGCGCTTT	ACTACGTCAT TGATGCAGTA	CTCCAGCGGC GAGGTCGCCG	CACACCCGGC GTGTGGGCCG
27701	GCCAGCACCT CGGTCGTGGA	GTTGTCAGCG CAACAGTCGC	CCATTATGAG GGTAATACTC	CAAGGAAATT GTTCCTTTAA	CCCACGCCCT GGGTGCGGGA
27751	TGTACACCTC	AATGGTCGGT	GTTTACCCTG	TTGCGGCTGG AACGCCGACC	TCGACGGGTT
27801	CTGATGAGTT	GGGCTTATTT	GATGTACTCG	GCGGGACCCC CGCCCTGGGG	TGTACTATAG
27851	CCGGGTCAAC GGCCCAGTTG	GGAATACGCG CCTTATGCGC	CCCACCGAAA GGGTGGCTTT	CCGAATTCTC GGCTTAAGAG	CTGGAACAGG GACCTTGTCC
27901	GCCGATAATG	GTGGTGTGGA	GCATTATTGG	TTAATCCCCG AATTAGGGGC	ATCAACCGGG
27951	CGACGGGACC	ACATGGTCCT	TTCAGGGCGA	CCCACCACTG GGGTGGTGAC	ACCATGAAGG
28001	GTCTCTGCGG	GTCCGGCTTC	AAGICTACTG	TAACTCAGGG ATTGAGTCCC	CGCGTCGAAC
28051	GCCCGCCGAA	AGCAGTGTCC	CACGCCAGCG	CCGGGCAGGG GGCCCGTCCC	ATATTGAGTG
28101	GACTGTTAGT	CTCCCGCTCC	ATAAGTCGAG	AACGACGAGT TTGCTGCTCA	GCCACTCGAG
28151	GAGCGAACCA	GAGGCAGGCC	TGCCCTGTAA	•	CCGCGGCCGG
28201	CGAGAAGTAA	GTGCGGAGCA	GTCCGTTAGG	ATTGAGACGT	GACCTCGTCC CTGGAGCAGG
•	AGACTCGGCG	CGAGACCTCC	GTAACCTTGA	GACGTTAAAT	TTGAGGAGTT AACTCCTCAA
	ACACGGTAGC	CAGATGAAAT	TGGGGAAGAG	CCCTGGAGGG	GGCCACTATC CCGGTGATAG
	GCCTAGTTAA	ATAAGGATTG	AAACTGCGCC	ATTTCCTGAG	GGCGGACGGC CCGCCTGCCG
28401	TACGACTGAA ATGCTGACTT	TGTTAAGTGG ACAATTCACC	AGAGGCAGAG TCTCCGTCTC	CAACTGCGCC GTTGACGCGG	TGAAACACCT ACTTTGTGGA

Figure 26 AD

28451	GGTCCACTGT CCAGGTGACA			CCGCGACTCC GGCGCTGAGG	
28501				AGGGCCCGGC TCCCGGGCCG	
28551				AGCCTGATTC TCGGACTAAG	
28601				GGGACCCTGT CCCTGGGACA	
28651				ATCAAGATCT TAGTTCTAGA	
28701				TAAAATATAC ATTTTATATG	
28751				CCCGCCCAAG GGGCGGGTTC	
28801				CCCTCTGTGA GGGAGACACT	
28851				GAACCTCTCC CTTGGAGAGG	
28901				CCTGCCGGGA GGACGGCCCT	
28951			ACACCTACCG	CCTGACCGTA GGACTGGCAT	
29001				CCAGAACAGG GGTCTTGTCC	
29051				GCAGCTACTG CGTCGATGAC	
29101				TAATTCAGGT ATTAAGTCCA	
29151				TTCTCTTTAT AAGAGAAATA	
29201	ACGCTTCTCT TGCGAAGAGA			TGTGTGCACA ACACACGTGT	
29251	TTGTCAGCTT AACAGTCGAA			CCCAAGATGA GGGTTCTACT	
29301	AATCCTAGGT	TTACTCACCC	TTGCGTCAGC	CCACGGTACC	ACCCAAAAGG
	TTAGGATCCA		AACGCAGTCG	GGTGCCATGG	TGGGTTTTCC

Figure 26 AE

29401	-	GAGAATATTT	TACGTGGTGT	CTTGTACTTT	TCGACGAATA
29451	TCGCCACAAA	AACAAAATTG	GCAAGTATGC CGTTCATACG	TGTTTATGCT	ATTTGGCAGC
29501	CAGGTGACAC	TACAGAGTAT	AATGTTACAG TTACAATGTC	TTTTCCAGGG	TAAAAGTCAT
				•	
29551	AAAACTTTTA	TGTATACTTT	TCCATTTTAT AGGTAAAATA	GAAATGTGCG	ACATTACCAT TGTAATGGTA
	IIIIGAAA	MCM111CHES.			
29601	GTACATGAGC	AAACAGTATA	AGTTGTGGCC	CCCACAAAAT	TGTGTGGAAA
			TCAACACCGG		
29651	ACACTGGCAC	TTTCTGCTGC	ACTGCTATGC	TAATTACAGT	GCTCGCTTTG
	TGTGACCGTG	AAAGACGACG	TGACGATACG	ATTAATGTCA	CGAGCGAAAC
29701	GTCTGTACCC	TACTCTATAT	TAAATACAAA	AGCAGACGCA	GCTTTATTGA
	CAGACATGGG	ATGAGATATA	ATTTATGTTT	TCGTCTGCGT	CGAAATAACT
29751	GGAAAAGAAA	ATGCCTTAAT	TTACTAAGTT	ACAAAGCTAA	TGTCACCACT
	CCTTTTCTTT	TACGGAATTA	AATGATTCAA	TGTTTCGATT	ACAGTGGTGA
29801	AACTGCTTTA	CTCGCTGCTT	GCAAAACAAA	TTCAAAAAGT	TAGCATTATA
<b>!</b>	TTGACGAAAT	GAGCGACGAA	CGTTTTGTTT	AAGTTTTTCA	ATCGTAATAT
29851	ATTAGAATAG	GATTTAAACC	CCCCGGTCAT	TTCCTGCTCA	ATACCATTCC
	TAATCTTATC	CTAAATTTGG	GGGGCCAGTA	AAGGACGAGT	TATGGTAAGG
29901	CCTGAACAAT	TGACTCTATG	TGGGATATGC	TCCAGCGCTA	CAACCTTGAA
			ACCCTATACG		
29951	GTCAGGCTTC	CTGGATGTCA	GCATCTGACT	TTGGCCAGCA	CCTGTCCCGC
			CGTAGACTGA		
30001	GGATTTGTTC	CAGTCCAACT	ACAGCGACCC	ACCCTAACAG	AGATGACCAA
	CCTAAACAAG	GTCAGGTTGA	TGTCGCTGGG	TGGGATTGTC	TCTACTGGTT
30051	CACAACCAAC	ececcecce	CTACCGGACT	TACATCTACC	ACAAATACAC
	GTGTTGGTTG	Cecceecec	GATGGCCTGA	ATGTAGATGG	TGTTTATGTG
30101	CCCAAGTTTC	TECCTTTETC	AATAACTGGG	ATAACTTGGG	CATGTGGTGG
	GGGTTCAAAG	ACGGAAACAG	TTATTGACCC	TATTGAACCC	GTACACCACC
30151	TTCTCCATAG	CGCTTATGTT	TGTATGCCTT	ATTATTATGT	GGCTCATCTG
	AAGAGGTATC	GCGAATACAA	ACATACGGAA	TAATAATACA	CCGAGTAGAC
30201	CTGCCTAAAG	CGCAAACGCG	CCCGACCACC	CATCTATAGT	CCCATCATTG
	GACGGATTTC	GCGTTTGCGC	GGGCTGGTGG	GTAGATATCA	GGGTAGTAAC
30251	TGCTACACCC	AAACAATGAT	GGAATCCATA	GATTGGACGG	ACTGAAACAC
	ACGATGTGGG	TTTGTTACTA	CCTTAGGTAT	CTAACCTGCC	TGACTTTGTG
30301	ATGTTCTTTT	CTCTTACAGT	ATGATTAAAT	GAGACATGAT	TCCTCGAGTT
	TACAAGAAAA	GAGAATGTCA	TACTAATTTA	CTCTGTACTA	AGGAGCTCAA

Figure 26 AF

30351	TTTATATTAC AAATATAATG	T CCTTGT ACTGGGAACA		
30401	TGCGGTTTCT ACGCCAAAGA	CACATCGAAG GTGTAGCTTC		
30451	TGCTTTACGG ACGAAATGCC	ATTTGTCACC TAAACAGTGG		
30501		TTATCCAGTG AATAGGTCAC		
30551	TCTCAGACAC AGAGTCTGTG	CATCCCCAGT GTAGGGGTCA		
30601		ATTATGAAAT TAATACTTTA		
30651		GTTTTGTTCC CAAAACAAGG		
30701		CTCGTATATG GAGCATATAC		
30751		GAAGCCTGGT CTTCGGACCA	 	
30801		CTTAGCCCTA GAATCGGGAT		
30851		ATGCCATGAA TACGGTACTT		
30901		CAAGTTGTTG GTTCAACAAC		
30951		TCCCACCCCC AGGGTGGGGG		
31001		GACACCCTAG CTGTGGGATC		
31051		AGAAAGACGC TCTTTCTGCG		
31101	CAAGAGCTCC GTTCTCGAGG	AAGACATGGT TTCTGTACCA		
31151	TTGTCTCGTA AACAGAGCAT	AAGCAGGCCA TTCGTCCGGT		
31201	ACCGCCTTAG TGGCGGAATC	CTACAAGTTG GATGTTCAAC		
31251	GTGGGAGAAA CACCCTCTTT			AAACCGAAGG TTTGGCTTCC

Figure 24 AG

31301	CTGCATTCAC	TCTTGTC	AAGGACCTGA	GGATCTCTGC	ACCCTT TA
	GACGTAAGTG	AGTGGAACAG	TTCCTGGACT	CCTAGAGACG	TGGGAALAAT
31351	AGACCCTGTG	CGGTCTCAAA	GATCTTATTC	CCTTTAACTA	AAAAAAAA
	TCTGGGACAC	GCCAGAGTTT	CTAGAATAAG	GGAAATTGAT	TTTTTTTTAT
31401	ATAATAAAGC	ATCACTTACT	TAAAATCAGT	TAGCAAATTT	CTGTCCAGTT
	TATTATTTCG	TAGTGAATGA	ATTTTAGTCA	ATCGTTTAAA	GACAGGTCAA
31451	TATTCAGCAG ATAAGTCGTC	CACCTCCTTG GTGGAGGAAC	CCCTCCTCCC	AGCTCTGGTA TCGAGACCAT	TTGCAGCTTC AACGTCGAAG
31501	CTCCTGGCTG	CAAACTTTCT	CCACAATCTA	AATGGAATGT	CAGTTTCCTC
	GAGGACCGAC	GTTTGAAAGA	GGTGTTAGAT	TTACCTTACA	GTCAAAGGAG
31551	CTGTTCCTGT	CCATCCGCAC	CCACTATCTT	CATGTTCTTG	CAGATGAAGC
	GACAAGGACA	GGTAGGCGTG	GGTGATAGAA	GTACAACAAC	GTCTACTTCG
31601	GCGCAAGACC	GTCTGAAGAT	ACCTTCAACC	CCGTGTATCC	ATATGACACG
	CGCGTTCTGG	CAGACTTCTA	TGGAAGTTGG	GGCACATAGG	TATACTGTGC
31651	GAAACCGGTC	CTCCAACTGT	GCCTTTTCTT	ACTCCTCCCT	TTGTATCCCC
	CTTTGGCCAG	GAGGTTGACA	CGGAAAAGAA	TGAGGAGGGA	AACATAGGGG
.31701	CAATGGGTTT	CAAGAGAGTC	CCCCTGGGGT	ACTCTCTTTG	CGCCTATCCG
	GTTACCCAAA	GTTCTCTCAG	GGGGACCCCA	TGAGAGAAAC	GCGGATAGGC
31751	AACCTCTAGT	TACCTCCAAT	GGCATGCTTG	CGCTCAAAAT	GGGCAACGGC
	TTGGAGATCA	ATGGAGGTTA	CCGTACGAAC	GCGAGTTTTA	CCCGTTGCCG
31801	GAGAGAGACC	ACGAGGCCGG TGCTCCGGCC	GTTGGAATGG	AGGGTTTTAC	ATTGGTGACA
31851	GAGCCCACCT	CTCAAAAAAA	CCAAGTCAAA	CATAAACCTG	GAAATATCTG
	CTCGGGTGGA	GAGTTTTTTT	GGTTĆAGTTT	GTATTTGGAC	CTTTATAGAC
31901	CACCCCTCAC	AGTTACCTCA	GAAGCCCTAA	CTGTGGCTGC	CGCCGCACCT
	GTGGGGAGTG	TCAATGGAGT	CTTCGGGATT	GACACCGACG	GCGGCGTGGA
31951	CTAATGGTCG	CGGGCAACAC	ACTCACCATG	CAATCACAGG	CCCCGCTAAC
	GATTACCAGC	GCCCGTTGTG	TGAGTGGTAC	GTTAGTGTCC	GGGGCGATTG
32001	CGTGCACGAC	TCCAAACTTA	GCATTGCCAC	CCAAGGACCC	CTCACAGTGT
	GCACGTGCTG	AGGTTTGAAT	CGTAACGGTG	GGTTCCTGGG	GAGTGTCACA
		CGATCGGGAC	GTTTGTAGTC	CGGGGGAGTG	GTGGTGGCTA
32101	AGCAGTACCC	TTACTATCAC	TGCCTCACCC	CCTCTAACTA	CTGCCACTGG
	TCGTCATGGG	AATGATAGTG	ACGGAGTGGG	GGAGATTGAT	GACGGTGACC
32151	TAGCTTGGGC	ATTGACTTGA	AAGAGCCCAT	TTATACACAA	AATGGAAAAC
	ATCGAACCCG	TAACTGAACT	TTCTCGGGTA	AATATGTGTT	TTACCTTTTG
32201	TAGGACTAAA	GTACGGGGCT	CCTTTGCATG	TAACAGACGA	CCTAAACACT
	ATCCTGATTT	CATGCCCCGA	GGAAACGTAC	ATTGTCTGCT	GGATTTGTGA

Figure 26 AH

32251			AGGTGTGACT TCCACACTGA	
32301			TGGGTTTTGA ACCCAAAACT	 
32351			AGGATTGATT TCCTAACTAA	
32401			TGATGCTCAA ACTACGAGTT	
32451	AGGACAGGGC TCCTGTCCCG		TAAACTCAGC ATTTGAGTCG	 
32501			TTTACAGCTT AAATGTCGAA	 CAAAAAGCTT GTTTTTCGAA
32551			CAAGGGGTTG GTTCCCCAAC	 
32601		•	GGCTTGAATT CCGAACTTAA	 
32651			AAAATTGGCC TTTTAACCGG	 
32701			ACTAGGAACT TGATCCTTGA	 
32751			'ACAAAAATAA TGTTTTTATT	 
32801			AACTGTAGAC TTGACATCTG	 -
32851			AAAATGTGGC TTTTACACCG	
32901			GCAGTTTGGC CGTCAAACCG	
32951			AGATTTGACG TCTAAACTGC	
33001	AATTCCTTCC TTAAGGAAGG		ATATTGGAAC TATAACCTTG	 
33051	TGAAGGCACA ACTTCCGTGT		ACGCTGTTGG TGCGACAACC	
33101	CTTATCCAAA GAATAGGTTT		AAAACTGCCA TTTTGACGGT	
33151	GTTTACTTAA CAAATGAATT		AACTAAACCT TTGATTTGGA	

Figure 26 AI

-					
33201	AAACGGTACA TTTGCCATGT	GACTTTGTC	GAGACACAAC CTCTGTGTTG	AZƏTƏACƏT AZƏTTƏƏA	TACTOT T ATGAGACA
33251				ACATTAATGA	
	GTAAAAGTAC	CCTGACCAGA	CCGGTGTTGA	TGTAATTACT	TTATAAACGG
33301				CAAGAATAAA	
	TGTAGGAGAA	TGTGAAAAAG	TATGTAACGG	GTTCTTATTT	CTTAGCAAAC
33351	TGTTATGTTT	ር አ አ ርርጥር ጥጥጥ	<u>አጥተጥተጥር አ</u> አጥ	TGCAGAAAAT	TTCAAGTCAT
				ACGTCTTTTA	
33401	TTTTCATTCA	CTACTATACC	CCCACCACCA	САТАССТТАТ	ACAGATCACC
22401				GTATCGAATA	
33451				ATTCAACCTG	
	_			TAAGTTGGAC	
33501	CCCAACACAC	AGAGTACACA	GTCCTTTCTC	CCCGGCTGGC	CTTAAAAAGC
	GGGTTGTGTG	TCTCATGTGT	CAGGAAAGAG	GGGCCGACCG	GAATTTTTCG
33551	<b>АТСАТАТСАТ</b>	GGGTAACAGA	CATATTCTTA	GGTGTTATAT	TCCACACGGT
				CCACAATATA	
33601	TTCCTGTCGA	GCCAAACGCT	CATCAGTGAT	ATTAATAAAC	TCCCCGGGCA
	AAGGACAGCT	CGGTTTGCGA	GTAGTCACTA	TAATTATTTG	AGGGCCCGT
33651	GCTCACTTAA	GTTCATGTCG	CTGTCCAGCT	GCTGAGCCAC	AGGCTGCTGT
				CGACTCGGTG	
33701				GGAGAAGTCC	
	GGTTGAACGC	CAACGAATTG	CCCGCCGCTT	CCTCTTCAGG	TGCGGATGTA
33751	GGGGGTAGAG	TCATAATCGT	GCATCAGGAT	AGGGCGGTGG	TGCTGCAGCA
••••				TCCCGCCACC	
33801				CCGTCCTGCA	
	CGCGCGCTTA	TTTGACGACG	GCGGCGGCGA	GGCAGGACGT	CCTTATGTTG
33851	*******************************	<b>שבישכיבי</b> א כיכ	CATICATION	ACCGCCCGCA	CCATABGGCG
33631				TGGCGGGCGT	
	INCCOLUNCE	AGAGGAG1CG	CINCILLOCO		
33901	CCTTGTCCTC	CGGGCACAGC	AGCGCACCCT	GATCTCACTT	AAATCAGCAC
	GGAACAGGAG	GCCCGTGTCG	TCGCGTGGGA	CTAGAGTGAA	TTTAGTCGTG
22051	AGTAACTGCA	CCACACCACC	ייבאייבייבייביי	TCARARTCCC	ACAGTGCAAG
33331	TO A THE ACT	CETETCETE	TGTTATAACA	AGTTTTAGGG	TGTCACGTTC
	ICAIIGACGI	C810100100			2010000
34001	GCGCTGTATC	CAAAGCTCAT	GGCGGGGACC	ACAGAACCCA	CGTGGCCATC
	CGCGACATAG	GTTTCGAGTA	CCGCCCCTGG	TGTCTTGGGT	GCACCGGTAG
34051	ATACCACAAG	CGCAGGTAGA	TTAAGTGGCG	ACCCCTCATA	AACACGCTGG
	TATGGTGTTC	GCGTCCATCT	AATTCACCGC	TGGGGAGTAT	TTGTGCGACC
34301	ACATAAACAT	TACCTCTTTT	GGCATGTTGT	AATTCACCAC	CTCCCGGTAC
34101	ጥርጥልጥጥርጥል	ATGGAGAAAA	CCGTACAACA	TTAAGTGGTG	GAGGGCCATG

Figure 26 AJ

34151	CATATAAACC GTATATTTGG	T CATTAAA A CTAATTT	CATGGCGCCA GTACCGCGGT	TCCACCACCAT AGGTGGTGGT	TCCTAA A A A AGGATT ST
34201				CTGCAGGGAA GACGTCCCTT	
34251				AACCATGGAT TTGGTACCTA	
34301				CACACGTGCA GTGTGCACGT	
34351				CATATCCCAG GTATAGGGTC	
34401				AGGGAAGACC TCCCTTCTGG	
34451		_		TCGGGCAGCA AGCCCGTCGT	
34501				AAAAGGAGGT TTTTCCTCCA	
34551				ATCGTGTTGG TAGCACAACC	
34601				TTTCCTGAAG AAAGGACTTC	
34651		•		GGTCTCGCCG CCAGAGCGGC	
34701				CTCAAAGCAT GAGTTTCGTA	
34751				ATGCGCCGCT TACGCGGCGA	
34801	CATCCACCAC GTAGGTGGTG			GCCAACCTAC CGGTTGGATG	
34851				GCTGGAAGAA CGACCTTCTT	
34901	TTTTTTATTC AAAAAATAAG			CAAAATGAAG GTTTTACTTC	
34951	TGAACGCGCT ACTTGCGCGA			AACTCTACAG TTGAGATGTC	
35001	GATAATGGCA CTATTACCGT			GGCTTCCAAA CCGAAGGTTT	
35051	CCCTCACGTC GGGAGTGCAG			ACCCTTCAGG TGGGAAGTCC	

Figure 26 AK

35101	TCTATAAACA AGATATTTGT	AAGGTCGTGG	TTCAACCATG AAGTTGGTAC	CCCAAATAAT GGGTTTATTA	TCTCAT G AGAGTAGAGC
35151	GGTGGAAGAG	TTATATAGAG	ATTCGTTTAG	CCGAATATTA GGCTTATAAT	TCAGGCCGGT
35201		GACGAGGTCT	CGCGGGAGGT	GGAAGTCGGA	GTTCGTCGCT
35251	ATCATGATTG TAGTACTAAC			AGACCTGTAT TCTGGACATA	
35301		ATTGTTTTTA	TGGCGCTAGG	GCATCCAGGG	AAGCGTCCCG
35351	GTCGACTTGT	ATTAGCACGT	CCAGACGTGC	GACCAGCGCG CTGGTCGCGC	CGGTGAAGGG
35401	GCGGTCCTTG	GTACTGTTTT	CTTGGGTGTG	TGATTATGAC ACTAATACTG	TGCGTATGAG
35451	CCTCGATACG	ATTGGTCGCA	TCGGGGCTAC	TAAGCTTGTT ATTCGAACAA	CGTACCCGCC
35501	GCTATATTTT	ACGTTCCACG	ACGAGTTTTT	ATCAGGCAAA TAGTCCGTTT	CGGAGCGCGT
35551		GTGTAGCATC	AGTACGAGTA	CGTCTATTTC	CGTCCATTCG
35601	AGGCCTTGGT	GGTGTCTTTT	TCTGTGGTAA	TTTCTCTCAA AAAGAGAGTT	TGTACAGACG
35651	CCCAAAGACG	TATTTGTGTT	TTATTTATT	CAAAAAAACA GTTTTTTTGT	AAATTTGTAA
35701	TCTTCGGACA	GAATGTTGTC	CTTTTTGTTG	CCTTATAAGC GGAATATTCG	TATTCTGCCT
35751		CGGCCGCACT	GGCATTTTTT	TGACCAGTGG	CACTAATTTT
35801	TCGTGGTGGC	TGTCGAGGAG	CCAGTACAGG	GGAGTCATAA CCTCAGTATT	ACATTCTGAG
		AGTCCAACTA	AGTGTAGCCA	GTCACGATTT	TTCGCTGGCT
		CCCTTATGTA	TGGGCGTCCG	CATCTCTGTT	GTAATGTCGG
35951	CCCATAGGAG GGGTATCCTC	GTATAACAAA CATATTGTTT	ATTAATAGGA TAATTATCCT	GAGAAAAACA CTCTTTTTGT	CATAAACACC GTATTTGTGG
36001	TGAAAAACCC ACTTTTTGGG	TCCTGCCTAG AGGACGGATC	GCAAAATAGC CGTTTTATCG	ACCCTCCCGC TGGGAGGGCG	TCCAGAACAA AGGTCTTGTT

Figure 26 AL

36051	CATACAGCGC GTATGTCGCG		GCAGCCATAA CGTCGGTATT		
36101			ACACCACTCG TGTGGTGAGC		
36151			CAAGTGCAGA GTTCACGTCT		
36201			GTCCACAAA CAGGTGTTTT		
36251			AAAGCCAAAA TTTCGGTTTT		
36301			GTTACGTCAC CAATGCAGTG		
36351			TTACTCCGCC AATGAGGCGG		
36401			CACGTCACAA GTGCAGTGTT		
					PacI
36451			AAGGTATATT		
	ATAACCGAAG	TTAGGTTTTA	TTCCATATAA	TAACTACTAC	AATTAATTCT
36501			GCTGGATGGC CGACCTACCG		
36551			ATGCCCGCGT TACGGGCGCA		
36601			GGGACAGCTT CCCTGTCGAA		
36651			TGCTGGCGTT ACGACCGCAA		
36701			CGACGCTCAA GCTGCGAGTT		
36751	ACAGGACTAT TGTCCTGATA	AAAGATACCA TTTCTATGGT	GGCGTTTCCC CCGCAAAGGG	CCTGGAAGCT GGACCTTCGA	CCCTCGTGCG GGGAGCACGC
36801	CTCTCCTGTT GAGAGGACAA	CCGACCCTGC GGCTGGGACG	CGCTTACCGG GCGAATGGCC	ATACCTGTCC TATGGACAGG	GCCTTTCTCC CGGAAAGAGG
36851	CTTCGGGAAG GAAGCCCTTC	CGTGGCGCTT GCACCGCGAA	TCTCATAGCT AGAGTATCGA	CACGCTGTAG GTGCGACATC	GTATCTCAGT CATAGAGTCA
36901	TCGGTGTAGG AGCCACATCC	TCGTTCGCTC AGCAAGCGAG	CAAGCTGGGC GTTCGACCCG	TGTGTGCACG ACACACGTGC	AACCCCCGT TTGGGGGGCA

Figure 26 AM

36951	TCAGCCCGAC	rgcgcct	TATCCGGTAA	CTATCGTCTT	GAGTCO TC
	AGTCGGGCTG	GcgACGCGGA	ATAGGCCATT	GATAGCAGAA	CTCAGG. GG
37001	GCCATTCTGT	GCTGAATAGC	CCACTGGCAG GGTGACCGTC	GTCGGTGACC	ATTGTCCTAA
37051	TCGTCTCGCT	CCATACATCC	CGGTGCTACA GCCACGATGT	CTCAAGAACT	TCACCACCGG
37101	ATTGATGCCG	ATGTGATCTT	GGACAGTATT CCTGTCATAA	ACCATAGACG	CGAGACGACT
37151	TCGGTCAATG	GAAGCCTTTT	AGAGTTGGTA TCTCAACCAT	CGAGAACTAG	GCCGTTTGTT
37201	TGGTGGCGAC	CATCGCCACC	TTTTTTTGTT AAAAAAACAA	ACGTTCGTCG	TCTAATGCGC
37251	GTCTTTTTTT	CCTAGAGTTC	AAGATCCTTT TTCTAGGAAA	CTAGAAAAGA	TGCCCCAGAC
37301	ACGCTCAGTG	GAACGAAAAC	TCACGTTAAG	GGATTTTGGT	CATGAGATTA
	TGCGAGTCAC	CTTGCTTTTG	AGTGCAATTC	CCTAAAACCA	GTACTCTAAT
37351	TCAAAAAGGA	TCTTCACCTA	GATCCTTTTA	AATCAATCTA	AAGTATATAT
	AGTTTTTCCT	AGAAGTGGAT	CTAGGAAAAT	TTAGTTAGAT	TTCATATATA
37401	GAGTAAACTT	GGTCTGACAG	TTACCAATGC	TTAATCAGTG	AGGCACCTAT
	CTCATTTGAA	CCAGACTGTC	AATGGTTACG	AATTAGTCAC	TCCGTGGATA
37451	CTCAGCGATC	TGTCTATTTC	GTTCATCCAT	AGTTGCCTGA	CTCCCCGTCG
	GAGTCGCTAG	ACAGATAAAG	CAAGTAGGTA	TCAACGGACT	GAGGGGCAGC
37501	TGTAGATAAC	TACGATACGG	GAGGGCTTAC	CATCTGGCCC	CAGTGCTGCA
	ACATCTATTG	ATGCTATGCC	CTCCCGAATG	GTAGACCGGG	GTCACGACGT
37551	TACTATGGCG	CTCTGGGTGC	CTCACCGGCT GAGTGGCCGA	GGTCTAAATA	GTCGTTATTT
37601	CCAGCCAGCC	GGAAGGGCCG	AGCGCAGAAG	TGGTCCTGCA	ACTTTATCCG
	GGTCGGTCGG	CCTTCCCGGC	TCGCGTCTTC	ACCAGGACGT	TGAAATAGGC
37651	CCTCCATCCA	GTCTATTAAT	TGTTGCCGGG	AAGCTAGAGT	AAGTAGTTCG
	GGAGGTAGGT	CAGATAATTA	ACAACGGCCC	TTCGATCTCA	TTCATCAAGC
	GGTCAATTAT	CAAACGCGTT	GCAACAACGG	TAACGATGTC	GCATCGTGGT CGTAGCACCA
37751	GTCACGCTCG	TCGTTTGGTA	TGGCTTCATT	CAGCTCCGGT	TCCCAACGAT
	CAGTGCGAGC	AGCAAACCAT	ACCGAAGTAA	GTCGAGGCCA	AGGGTTGCTA
37801	CAAGGCGAGT	TACATGATCC	CCCATGTTGT	GCAAAAAAGC	GGTTAGCTCC
	GTTCCGCTCA	ATGTACTAGG	GGGTACAACA	CGTTTTTTCG	CCAATCGAGG
37851	TTCGGTCCTC	CGATCGTTGT	CAGAAGTAAG	TTGGCCGCAG	TGTTATCACT
	AAGCCAGGAG	GCTAGCAACA	GTCTTCATTC	AACCGGCGTC	ACAATAGTGA

Figure 26 AN

37901	CATGGTTATG GTACCAATAC	CGCCTGACG	ATAATTCTCT TATTAAGAGA	TACTGTCATG ATGACAGTAC	CCATCO A GGTAGGCATT
37951	GATGCTTTTC				
	CTACGAAAAG	ACACTGACCA	CTCATGAGTT	GGTTCAGTAA	GACTCTTATC
38001			CTCTTGCCCG		
	ACATACGCÇG	CTGGCTCAAC	GAGAACGGGC	CGCAGTTGTG	CCCTATTATG
38051			TAAAAGTGCT		
	GCGCGGTGTA	TCGTCTTGAA	ATTTTCACGA	GTAGTAACCT	TTTGCAAGAA
38101	CGGGGCGAAA				
•	GCCCCGCTTT	TGAGAGTTCC	TAGAATGGCG	ACAACTCTAG	GTCAAGCTAC
38151			CTGATCTTCA		
	ATTGGGTGAG	CACGTGGGTT	GACTAGAAGT	CGTAGAAAAT	GAAAGTGGTC
38201			CAGGAAGGCA		
	GCAAAGACCC	ACTCGTTTTT	GTCCTTCCGT	TTTACGGCGT	TTTTTCCCTT
38251			TGAATACTCA		
	ATTCCCGCTG	TGCCTTTACA	ACTTATGAGT	atgagaagga	AAAAGTTATA
38301			TTATTGTCTC		
	ATAACTTCGT	AAATAGTCCC	AATAACAGAG	TACTCGCCTA	TGTATAAACT
38351			AAATAGGGGT		
	TACATAAATC	TTTTTATTTG	TTTATCCCCA	AGGCGCGTGT	AAAGGGGCTT
38401	AAGTGCCACC				
	TTCACGGTGG	ACTGCAGATT	CTTTGGTAAT	AATAGTACTG	TAATTGGATA
38451	AAAAATAGGC				
	TTTTTATCCG	CATAGTGCTC	CGGGAAAGCA	GAAGTTCTTA	ACCTAGGCTT
•		PacI			
38501	TTCTTAATTT	CTTAATTAA	(SEO ID NO:	32)	
-			(SEQ ID NO:	-	

Figure 26 AO

## MRKAd5nef MER1063 (MRKAd5 Pre-Adenoviral Vector Containing the G2A,LLA nef Coding Region)

1	CATCATCAAT	AATATACCTT	ATTITGGATT	GAAGCCAATA	TGATAATGAG
	GTAGTAGTTA	TTATATGGAA	TAAAACCTAA	CTTCGGTTAT	ACTATTACTC
51	GGGGTGGAGT CCCCACCTCA	TTGTGACGTG AACACTGCAC	CCCCCCCCC	TGGGAACGGG ACCCTTGCCC	GCGGGTGACG CGCCCACTGC
101	TAGTAGTGTG	GCGGAAGTGT	GATGTTGCAA	GTGTGGCGGA	ACACATGTAA
	ATCATCACAC	CGCCTTCACA	CTACAACGTT	CACACCGCCT	TGTGTACATT
151		TGGCAAAAGT ACCGTTTTCA			
201	GAAGTGACAA	TTTTCGCGCG	GTTTTAGGCG	GATGTTGTAG	TAAATTTGGG
	CTTCACTGTT	AAAAGCGCGC	CAAAATCCGC	CTACAACATC	ATTTAAACCC
251	CGTAACCGAG	TAAGATTTGG	CCATTTTCGC	GGGAAAACTG	AATAAGAGGA
	GCATTGGCTC	ATTCTAAACC	GGTAAAAGCG	CCCTTTTGAC	TTATTCTCCT
301	AGTGAAATCT	GAATAATTTT	GTGTTACTCA	TAGCGCGTAA	TATTTGTCTA
	TCACTTTAGA	CTTATTAAAA	CACAATGAGT	ATCGCGCATT	ATAAACAGAT
351	CCCGCCGCCCC	GACTTTGACC CTGAAACTGG	GTTTACGTGG CAAATGCACC	AGACTCGCCC TCTGAGCGGG	AGGTGTTTTT TCCACAAAAA
401	CTCAGGTGTT	TTCCGCGTTC	CGGGTCAAAG	TTGGCGTTTT	ATTATTATAG
	GAGTCCACAA	AAGGCGCAAG	GCCCAGTTTC	AACCGCAAAA	TAATAATATC
451	GCGGCCGCT	TCCATTGCAT AGGTAACGTA	ACGTTGTATC TGCAACATAG	CATATCATAA GTATAGTATT	TATGTACATT ATACATGTAA
501	TATATTGGCT	CATGTCCAAC	ATTACCGCCA	TGTTGACATT	GATTATTGAC
	ATATAACCGA	GTACAGGTTG	TAATGGCGGT	ACAACTGTAA	CTAATAACTG
551	TAGTTATTAA	TAGTAATCAA	TTACGGGGTC	ATTAGTTCAT	AGCCCATATA
	ATCAATAATT	ATCATTAGTT	AATGCCCCAG	TAATCAAGTA	TCGGGTATAT
601	TGGAGTTCCG	CGTTACATAA	CTTACGGTAA	ATGGCCCGCC	TGGCTGACCG
	ACCTCAAGGC	GCAATGTATT	GAATGCCATT	TACCGGGCGG	ACCGACTGGC
651	CCCAACGACC	CCCGCCCATT	GACGTCAATA	ATGACGTATG	TTCCCATAGT
	GGGTTGCTGG	GGGCGGGTAA	CTGCAGTTAT	TACTGCATAC	AAGGGTATCA
701	AACGCCAATA	GGGACTTTCC	ATTGACGTCA	ATGGCTGGAG	TATTTACGGT
	TTGCGGTTAT	CCCTGAAAGG	TAACTGCAGT	TACCCACCTC	ATAAATGCCA
751	AAACTGCCCA	CTTGGCAGTA	CATCAAGTGT	ATCATATGCC	AAGTACGCCC
	TTTGACGGGT	GAACCGTCAT	GTAGTTCACA	TAGTATACGG	TTCATGCGGG
801	CCTATTGACG	TCAATGACGG	TAAATGGCCC	GCCTGGCATT	ATGCCCAGTA
	GGATAACTGC	AGTTACTGCC	ATTTACCGGG	CGGACCGTAA	TACGGGTCAT

Figure 27A

851	CATGACCTTA GTACTGGAAT	TEACTTTC ACCTGAAAG	CTACTTGGCA GATGAACCGT	GTACATCTAC CATGTAGATG	GTATTA TA CATAAT AGT
. 901		CATGGTGATG GTACCACTAC			
951		ACTCACGGGG TGAGTGCCCC			
1001		TTTTGGCACC AAAACCGTGG			
1051		CCCATTGACG GGGTAACTGC			
1101		GCAGAGCTCG CGTCTCGAGC			
1151		TGTTTTGACC ACAAAACTGG			
1201		GGAACGGTGC CCTTGCCACG			
1251		ACCATGGCCG TGGTACCGGC			
1301		CAGGGAGAGG CTCCCTCTCC			
1351		CCGAGCCCGC GGCTCGGGCG			
1401		CACGGCGCCA GTGCCGCGGT			
1451		CTGGCTGGAG GACCGACCTC			
1501		AGGTGCCCCT TCCACGGGGA			
1551		TTCCTGAAGG AAGGACTTCC			
1601	CCCAGAAGAG GGGTCTTCTC	GCAGGACATC CGTCCTGTAG			
1651	TACTTCCCCG ATGAAGGGGC	ACTGGCAGAA TGACCGTCTT			
1701	CCTGACCTTC GGACTGGAAG	GGCTGGTGCT CCGACCACGA			
1751	TGGAGGAGGC ACCTCCTCCG	CAACGAGGGC GTTGCTCCCG			

Figure 27B

1801	CAGCACGGCA GTCGTGCCGT	TAGGACCC AGCTCCTGGG	CGAGAAGGAG GCTCTTCCTC	GTGCTGGAGT CACGACCTCA	GGAGGT A CCTCCAAGCT
1851				GGAGCTGCAC CCTCGACGTG	
1901				CTGTGCCTTC GACACGGAAG	
1951				TCCTTGACCC AGGAACTGGG	
2001				GGAAATTGCA CCTTTAACGT	
2051				GGGTGGGGCA CCCACCCCGT	
2101				GCTGGGGATG CGACCCCTAC	
2151				TGTGGGCGTG ACACCCGCAC	
2201				GTAGTTTTGT CATCAAAACA	
2251				CGTTTGATGG GCAAACTACC	
2301				TGGGCCGGGG	
2351				CGTCCTGCCC GCAGGACGGG	
2401				CGCCGTTGGA GCGGCAACCT	
2451				GCCCGCGGA CGGGCGCCT	
2501				TGCAGCTTCC ACGTCGAAGG	
2551	CCCGCGATGA GGGCGCTACT	CAAGTTGACG GTTCAACTGC	GCTCTTTTGG CGAGAAAACC	CACAATTGGA GTGTTAACCT	TTCTTTGACC AAGAAACTGG
2601	CGGGAACTTA GCCCTTGAAT	ATGTCGTTTC TACAGCAAAG	TCAGCAGCTG AGTCGTCGAC	TTGGATCTGC AACCTAGACG	GCCAGCAGGT CGGTCGTCCA
2651	TTCTGCCCTG AAGACGGGAC	AAGGCTTCCT TTCCGAAGGA	CCCCTCCCAA GGGGAGGGTT	TGCGGTTTAA ACGCCAAATT	AACATAAATA TTGTATTTAT
2701	AAAAACCAGA TTTTTGGTCT				TTGCTGTCTT AACGACAGAA

Figure 27C

2751	TATTTAGGGG ATAAATCCCC	TTTTGCGCGC AAAACGCGCG			
2801	GTTGAGGGTC CAACTCCCAG	CTGTGTATTT GACACATAAA			
2851		CATGGGCATA GTACCCGTAT			
2901		CATGCTGCGG GTACGACGCC			
2951		GCGTGGTGCC CGCACCACGG			
3001		CCCCTTGCTG CGGGAACCAC			
3051		GTGGGGATAT ÇACCCCTATA			
3101		CCAGCCATAT GGTCGGTATA			
3151		GTATCCGGTG CATAGGCCAC			
3201		GGAAGAACTT CCTTCTTGAA	•••••		
3251	CATGCATTCG GTACGTAAGC	TCCATAATGA AGGTATTACT			
3301		TCTGGGATCA AGACCCTAGT			
3351	AGCAGTATCC	CCATTTTTAC GGTAAAAATG	TTTCGCGCCC	GCCTCCCACG	GTCTGACGCC
3401		CCATCCGGCC GGTAGGCCGG			
3451		TTTGAGTTCA AAACTCAAGT			
3501					AAGAAAGCAG TTCTTTCGTC
3551	CTTCCTGAGC CAAGGACTCG				TAAATCACAC ATTTAGTGTG
3601	CTATTACCGG GATAATGGCC				GCCGTCATCC CGGCAGTAGG
3651					GCATGTTTTC CGTACAAAAG

Figure 270

3701	CCTGACCAAA GGACTGGTTT	CCAGAA AGGCGGTCTT	OCGCGAGCGG	GCCCAGCGAT CGGGTCGCTA	AGCAGT TT TCGTCAAGAA
3751	GCAAGGAAGC	AAAGTTTTTC	AACGGTTTGA	GACCGTCCGC	CGTAGGCATG
	CGTTCCTTCG	TTTCAAAAAG	TTGCCAAACT	CTGGCAGGCG	GCATCCGTAC
3801	CTTTTGAGCG	TTTGACCAAG	CAGTTCCAGG	CGGTCCCACA	GCTCGGTCAC
	GAAAACTCGC	AAACTGGTTC	GTCAAGGTCC	GCCAGGGTGT	CGAGCCAGTG
3851				TCCTCGTTTC AGGAGCAAAG	
3901	GCGGCTTTCG	CTGTACGGCA	GTAGTCGGTG	CTCGTCCAGA	CGGGCCAGGG
	CGCCGAAAGC	GACATGCCGT	CATCAGCCAC	GAGCAGGTCT	GCCCGGTCCC
3951	TCATGTCTTT	CCACGGGCGC	AGGGTCCTCG	TCAGCGTAGT	CTGGGTCACG
	AGTACAGAAA	GGTGCCCGCG	TCCCAGGAGC	AGTCGCATCA	GACCCAGTGC
4001	GTGAAGGGGT	GCGCTCCGGG	CTGCGCGCTG	GCCAGGGTGC	GCTTGAGGCT
	CACTTCCCCA	CGCGAGGCCC	GACGCGCGAC	CGGTCCCACG	CGAACTCCGA
4051	GGTCCTGCTG	GTGCTGAAGC	GCTGCCGGTC	TTCGCCCTGC	GCGTCGGCCA
	CCAGGACGAC	CACGACTTCG	CGACGGCCAG	AAGCGGGACG	CGCAGCCGGT
4101	GGTAGCATTT CCATCGTAAA	GACCATGGTG CTGGTACCAC	TCATAGTCCA AGTATCAGGT	CCCCCCCCC	GGCGTGGCCC CCGCACCGGG
4151 .	TTGGCGCGCA	GCTTGCCCTT	GGAGGAGGCG	CCGCACGAGG	GGCAGTGCAG
	AACCGCGCGT	CGAACGGGAA	CCTCCTCCGC	GGCGTGCTCC	CCGTCACGTC
4201	ACTTTTGAGG	GCGTAGAGCT	TGGGCGCGAG	AAATACCGAT	TCCGGGGAGT
	TGAAAACTCC	CGCATCTCGA	ACCCGCGCTC	TTTATGGCTA	AGGCCCCTCA
4251				TCTCGCATTC AGAGCGTAAG	
4301	GTGAGCTCTG	GCCGTTCGGG	GTCAAAAACC	AGGTTTCCCC	CATGCTTTTT
	CACTCGAGAC	CGGCAAGCCC	CAGTTTTTGG	TCCAAAGGGG	GTACGAAAAA
4351	GATGCGTTTC	TTACCTCTGG	TTTCCATGAG	CCGGTGTCCA	CGCTCGGTGA
	CTACGCAAAG	AATGGAGACC	AAAGGTACTC	GGCCACAGGT	GCGAGCCACT
4401	CGAAAAGGCT	GTCCGTGTCC	CCGTATACAG	ACTTGAGAGG	CCTGTCCTCG
	GCTTTTCCGA	CAGGCACAGG	GGCATATGTC	TGAACTCTCC	GGACAGGAGC
4451	AGCGGTGTTC	CGCGGTCCTC	CTCGTATAGA	AACTCGGACC	ACTCTGAGAC
	TCGCCACAAG	GCGCCAGGAG	GAGCATATCT	TTGAGCCTGG	TGAGACTCTG
4501	AAAGGCTCGC	GTCCAGGCCA	GCACGAAGGA	GGCTAAGTGG	GAGGGGTAGC
	TTTCCGAGCG	CAGGTCCGGT	CGTGCTTCCT	CCGATTCACC	CTCCCCATCG
4551	GGTCGTTGTC	CACTAGGGGG	TCCACTCGCT	CCAGGGTGTG	AAGACACATG
	CCAGCAACAG	GTGATCCCCC	AGGTGAGCGA	GGTCCCACAC	TTCTGTGTAC
4601	TCGCCCTCTT	CGGCATCAAG	GAAGGTGATT	GGTTTGTAGG	TGTAGGCCAC
	AGCGGGAGAA	GCCGTAGTTC	CTTCCACTAA	CCAAACATCC	ACATCCGGTG

Figure 27E

4651	GTGACCGGGT CACTGGCCCA	CAAGGACTTC	GGGGGCTATA CCCCCGATAT	AAAGGGGGTG TTTCCCCCAC	GGGGCC TT
4701				CGAGGGCCAG GCTCCCGGTC	
4751	GAGTACTCCC CTCATGAGGG			TCTGCGCTAA AGACGCGATT	
4801				CTGGCCCGCG GACCGGGCGC	
4851	TGAGGGTGGC ACTCCCACCG			AGACAATCTT TCTGTTAGAA	
4901				TTGGACAGCA AACCTGTCGT	
4951				GGCGCGCTCC CCGCGCGAGG	
5001 .	TGTTTAGCTG ACAAATCGAC			ACCGCCATTC TGGCGGTAAG	
5051				CGCCAACCGC GCGGTTGGCG	
5101				TCCGCGTAGG AGGCGCATCC	
5151				AGAATGGCGG TCTTACCGCC	
5201				ACGGTAAAGA TGCCATTTCT	
5251				TCCTTGCAAG AGGAACGTTC	
5301				CGTATGGGTT GCATACCCAA	CACTGGGGGA CTCACCCCT
5351				GCGTACATGC CGCATGTACG	CGCAAATGTC GCGTTTACAG
5401	GTAAACGTAG CATTTGCATC	AGGGGCTCTC TCCCCGAGAG	TGAGTATTCC ACTCATAAGG	AAGATATGTA TTCTATACAT	GGGTAGCATC CCCATCGTAG
5451					CTCCCAGGGA CACCCTCCCT
5501					CTGCTCGGAA GACGAGCCTT
5551					GTTGGACGCT CAACCTGCGA

Figure 27F

5601	GGAAGACGTT CCTTCTGCAA	CTTCGACCGC	TCTGTGAGAC AGACACTCTG	CTACCGCGTC GATGGCGCAG	ACGCACTCCTTC
5651	GAGGCGTAGG	agtegegeag	CTTGTTGACC	AGCTCGGCGG	TGACCTGCAC
	CTCCGCATCC	teagegegte	GAACAACTGG	TCGAGCCGCC	ACTGGACGTG
5701	GTCTAGGGCG	CAGTAGTCCA	GGGTTTCCTT	GATGATGTCA	TACTTATCCT
	CAGATCCCGC	GTCATCAGGT	CCCAAAGGAA	CTACTACAGT	ATGAATAGGA
5751	GTCCCTTTTT	TTTCCACAGC	TCGCGGTTGA	GGACAAACTC	TTCGCGGTCT
	CAGGGAAAAA	AAAGGTGTCG	AGCGCCAACT	CCTGTTTGAG	AAGCGCCAGA
5801				GCCTCCGAAC CGGAGGCTTG	
5851	TAGCATGTAG	AACTGGTTGA	CGGCCTGGTA	GGCGCAGCAT	CCCTTTTCTA
	ATCGTACATC	TTGACCAACT	GCCGGACCAT	CCGCGTCGTA	GGGAAAAGAT
5901	CGGGTAGCGC	GTATGCCTGC	GCGGCCTTCC	GGAGCGAGGT	GTGGGTGAGC
	GCCCATCGCG	CATACGGACG	CGCCGGAAGG	CCTCGCTCCA	CACCCACTCG
5951	GCAAAGGTGT	CCCTGACCAT	GACTTTGAGG	TACTGGTATT	TGAAGTCAGT
	CGTTTCCACA	GCGACTGGTA	CTGAAACTCC	ATGACCATAA	ACTTCAGTCA
6001	GTCGTCGCAT	CCGCCCTGCT	CCCAGAGCAA	AAAGTCCGTG	CGCTTTTTGG
	CAGCAGCGTA	GGCGGGACGA	GGGTCTCGTT	TTTCAGGCAC	GCGAAAAACC
6051	AACGCGGATT	TGGCAGGGCG	AAGGTGACAT	CGTTGAAGAG	TATCTTTCCC
	TTGCGCCTAA	ACCGTCCCGC	TTCCACTGTA	GCAACTTCTC	ATAGAAAGGG
6101	GCGCGAGGCA	TAAAGTTGCG	TGTGATGCGG	AAGGGTCCCG	GCACCTCGGA
	CGCGCTCCGT	ATTTCAACGC	ACACTACGCC	TTCCCAGGGC	CGTGGAGCCT
6151	ACGGTTGTTA	ATTACCTGGG	CGGCGAGCAC	GATCTCGTCA	AAGCCGTTGA
	TGCCAACAAT	TAATGGACCC	GCCGCTCGTG	CTAGAGCAGT	TTCGGCAACT
6201	TGTTGTGGCC	CACAATGTAA	AGTTCCAAGA	AGCGCGGGAT	GCCCTTGATG
	ACAACACCGG	GTGTTACATT	TCAAGGTTCT	TCGCGCCCTA	CGGGAACTAC
6251	GAAGGCAATT	TTTTAAGTTC	CTCGTAGGTG	AGCTCTTCAG	GGGAGCTGAG
	CTTCCGTTAA	AAAATTCAAG	GAGCATCCAC	TCGAGAAGTC	CCCTCGACTC
6301	CCCGTGCTCT	GAAAGGGCCC	AGTCTGCAAG	ATGAGGGTTG	GAAGCGACGA
	GGGCACGAGA	CTTTCCCGGG	TCAGACGTTC	TACTCCCAAC	CTTCGCTGCT
6351	ATGAGCTCCA	CAGGTCACGG	GCCATTAGCA	TTTGCAGGTG	GTCGCGAAAG
	TACTCGAGGT	GTCCAGTGCC	CGGTAATCGT	AAACGTCCAC	CAGCGCTTTC
6401	GTCCTAAACT	GGCGACCTAT	GGCCATTTTT	TCTGGGGTGA	TGCAGTAGAA
	CAGGATTTGA	CCGCTGGATA	CCGGTAAAAA	AGACCCCACT	ACGTCATCTT
6451	GGTAAGCGGG	TCTTGTTCCC	AGCGGTCCCA	TCCAAGGTTC	GCGGCTAGGT
	CCATTCGCCC	AGAACAAGGG	TCGCCAGGGT	AGGTTCCAAG	CGCCGATCCA
6501	CTCGCGCGGC	AGTCACTAGA	GGCTCATCTC	CGCCGAACTT	CATGACCAGC
	GAGCGCGCCG	TCAGTGATCT	CCGAGTAGAG	GCGGCTTGAA	GTACTGGTCG

Figure 27G

6551	ATGAAGGGCA	CTGCTT	CCCAAAGGCC	CCCATCCAAG GGGTAGGTTC	TATACC CAGAG
6601	TACATCGTAG	GTGACAAAGA	GACGCTCGGT	GCGAGGATGC	GAGCCGATCG
		•		CGCTCCTACG	
6651				AGGAGTGGCT TCCTCACCGA	
6701				CACTCGTGCT GTGAGCACGA	
6751	AAAACGTGCG TTTTGCACGC	CAGTACTGGC GTCATGACCG	AGCGGTGCAC TCGCCACGTG	GGGCTGTACA CCCGACATGT	TCCTGCACGA AGGACGTGCT
6801	GGTTGACCTG CCAACTGGAC	ACGACCGCGC TGCTGGCGCG	ACAAGGAAGC TGTTCCTTCG	AGAGTGGGAA TCTCACCCTT	TTTGAGCCCC AAACTCGGGG
6851	TCGCCTGGCG AGCGGACCGC	GGTTTGGCTG CCAAACCGAC	GTGGTCTTCT CACCAGAAGA	ACTTCGGCTG TGAAGCCGAC	CTTGTCCTTG GAACAGGAAC
6901				GGATCGGACC CCTAGCCTGG	
6951				GCGGTCGGAG CGCCAGCCTC	
7001				TGGAGCTCCC ACCTCGAGGG	
7051				GCATAGACGG CGTATCTGCC	
7101	GGGCTAGATC CCCGATCTAG	CAGGTGATAC GTCCACTATG	CTAATTTCCA GATTAAAGGT	GGGGCTGGTT CCCCGACCAA	GGTGGCGGCG CCACCGCCGC
7151				GGCGCGACTA CCGCGCTGAT	CGGTACCGCG GCCATGGCGC
7201	0000000000	TGGGCCGCGC	GGGTGTCCTT CCCACAGGAA	GGATGATGCA CCTACTACGT	TCTAAAAGCG AGATTTTCGC
7251	GTGACGCGGG CACTGCGCCC	CGAGCCCCCG GCTCGGGGGC	GAGGTAGGGG CTCCATCCCC	GGGCTCCGGA CCCGAGGCCT	CCCGCCGGGA
7301	GAGGGGGCAG CTCCCCCGTC	GGGCACGTCG CCCGTGCAGC	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	CGGGCAGGAG	CTGGTGCTGC GACCACGACG
7351	GCGCGTAGGT CGCGCATCCA	TGCTGGCGAA ACGACCGCTT	CGCGACGACG GCGCTGCTGC	CGGCGGTTGA GCCGCCAACT	TCTCCTGAAT AGAGGACTTA
7401	CTGGCGCCTC GACCGCGGAG	TGCGTGAAGA ACGCACTTCT	CGACGGGCCC GCTGCCCGGG	GGTGAGCTTG CCACTCGAAC	AACCTGAAAG TTGGACTTTC
7451	AGAGTTCGAC TCTCAAGCTG	AGAATCAATT TCTTAGTTAA	TCGGTGTCGT AGCCACAGCA	TGACGGCGGC ACTGCCGCCG	CTGGCGCAAA GACCGCGTTT

Figure 27H

7501	ATCTCCTGCA TAGAGGACGT	CENTROTGA GAGGACT	GTTGTCTTGA CAACAGAACT	TAGGCGATON ATCCGCTAGA	GCCGGT. T
7551		TCTTCCTCCT AGAAGGAGGA			
7601		GTCGTTGGAA CAGCAACCTT			
7651		CGTTCCAGAC GCAAGGTCTG			
7701		ATGACCACCT TACTGGTGGA			
7751	AGACGGCGTA	GTTTCGCAGG	CGCTGAAAGA	GGTAGTTGAG	GGTGGTGGCG
	TCTGCCGCAT	CAAAGCGTCC	GCGACTTTCT	CCATCAACTC	CCACCACCGC
7801	GTGTGTTCTG	CCACGAAGAA	GTACATAACC	CAGCGTCGCA	ACGTGGATTC
	CACACAAGAC	GGTGCTTCTT	CATGTATTGG	GTCGCAGCGT	TGCACCTAAG
7851	GTTGATATCC	CCCAAGGCCT	CAAGGCGCTC	CATGGCCTCG	TAGAAGTCCA
	CAACTATAGG	GGGTTCCGGA	GTTCCGCGAG	GTACCGGAGC	ATCTTCAGGT
7901		GAAAAACTGG CTTTTTGACC			
7951	TCCAGAAGAC	GGATGAGCTC	GGCGACAGTG	TCGCGCACCT	CGCGCTCAAA
	AGGTCTTCTG	CCTACTCGAG	CCGCTGTCAC	AGCGCGTGGA	GCGCGAGTTT
8001	GGCTACAGGG	GCCTCTTCTT	CTTCTTCAAT	CTCCTCTTCC	ATAAGGGCCT
	CCGATGTCCC	CGGAGAAGAA	GAAGAAGTTA	GAGGAGAAGG	TATTCCCGGA
8051	CCCCTTCTTC	TTCTTCTGGC	GGCGGTGGGG	GAGGGGGGAC	ACGGCGGCGA
	GGGGAAGAAG	AAGAAGACCG	CCGCCACCCC	CTCCCCCTG	TGCCGCCGCT
8101	CGACGGCGCA	CCGGGAGGCG	GTCGACAAAG	CGCTCGATCA	TCTCCCCGCG
	GCTGCCGCGT	GGCCCTCCGC	CAGCTGTTTC	GCGAGCTAGT	AGAGGGGCGC
8151		ATGGTCTCGG TACCAGAGCC			
8201		GCCGCCCGTC CGGCGGGCAG			
8251	CCATGCGGCA	GGGATACGGC	GCTAACGATG	CATCTCAACA	ATTGTTGTGT
	GGTACGCCGT	CCCTATGCCG	CGATTGCTAC	GTAGAGTTGT	TAACAACACA
8301	AGGTACTCCG	CCGCCGAGGG	ACCTGAGCGA	GTCCGCATCG	ACCGGATCGG
	TCCATGAGGC	GGCGGCTCCC	TGGACTCGCT	CAGGCGTAGC	TGGCCTAGCC
B351	AAAACCTCTC	GAGAAAGGCG	TCTAACCAGT	CACAGTCGCA	AGGTAGGCTG
	TTTTGGAGAG	CTCTTTCCGC	AGATTGGTCA	GTGTCAGCGT	TCCATCCGAC
8401	AGCACCGTGG TCGTGGCACC	CGGGCGGCAG GCCCGCCGTC	CCCCCCCCC	TCGGGGTTGT AGCCCCAACA	TTCTGGCGGA AAGACCGCCT

Figure 27I

8451		ATGTAAT TACTACATTA			CCCCCTACC
8501		CACCATGTCC GTGGTACAGG			
8551	TCGGCCATGC AGCCGGTACG	CCCAGGCTTC GGGTCCGAAG			
8601		AGCCTTTCTA TCGGAAAGAT		-	
8651		TGCATCTATC ACGTAGATAG	GCTGCGGCGG		TGGCCGTAGG ACCGGCATCC
8701	ACCGCGGGAG	AAGGAGGGTA	CGCACACTGG	GCCTTCGGGG	
8751		AGGTCGCCGA TCCAGCCGCT			GCCTGCTGCA CGGACGACGT
8801					GCGGTGGTAT CGCCACCATA
8851					ACCAGTTAAC TGGTCAATTG
8901	GGTCTGGTGA CCAGACCACT	CCCGGCTGCG	AGAGCTCGGT TCTCGAGCCA	GTACCTGAGA CATGGACTCT	CGCGAGTAAG GCGCTCATTC
8951	CCCTCGAGTC GGGAGCTCAG	AAATACGTAG TTTATGCATC	TCGTTGCAAG AGCAACGTTC	TCCGCACCAG AGGCGTGGTC	GTACTGGTAT CATGACCATA
9001					AGCGTAGGGT TCGCATCCCA
9051					TGATATCCGT ACTATAGGCA
9101					GGAGGCGCGC CCTCCGCGCG
9151	CCTTTCAGCG	CCTGCGCCAA	GGTCTACAAC	GCGTCGCCGT	AAAAGTGCTC TTTTCACGAG
9201	CATGGTCGGG GTACCAGCCC	ACGCTCTGGC TGCGAGACCG	CGGTCAGGCG GCCAGTCCGC	CGCGCAATCG GCGCGTTAGC	TTGACGCTCT AACTGCGAGA
9251	AGACCGTGCA TCTGGCACGT	AAAGGAGAGC TTTCCTCTCG	CTGTAAGCGG GACATTCGCC	GCACTCTTCC CGTGAGAAGG	CTCGTCTGGT
9301	GGATAAATTC CCTATTTAAG	GCAAGGGTAT CGTTCCCATA	CATGGCGGAC GTACCGCCTG	GACCGGGGTT CTGGCCCCAA	CGAGCCCCGT GCTCGGGGCA
9351	ATCCGGCCGT TAGGCCGGCA	CCGCCGTGAT GGCGGCACTA	CCATGCGGTT GGTACGCCAA	ACCGCCCGCG TGGCGGGGGCGC	TGTCGAACCC ACAGCTTGGG

Figure 27J

9401	AGGTGTGCGA TCCACACGCT				CTTCCT A GAAGGAAGGT
9451	CCCCCCCCCC	CTGCTGCGCT GACGACGCGA		-	
9501	TAAGCGGTTA ATTCGCCAAT	GGCTGGAAAG CCGACCTTTC			
9551		ATTTTCCAAG TAAAAGGTTC			
9601	TCGGACCGGC AGCCTGGCCG	CGGACTGCGG GCCTGACGCC			
9651		GCAAATTCCT CGTTTAAGGA			
9701	TTTCCCAGAT AAAGGGTCTA	GCATCCGGTG CGTAGGCCAC			
9751		AAGAGCAGCG TTCTCGTCGC			
9801	TACCGCGTCA ATGGCGCAGT	GGAGGGGCGA CCTCCCGGCT		- <del>-</del>	- <del>-</del>
9851		CCCCCCCCCC			
9901		TGGCGCGGCT ACCGCGCCGA			
9951		AAGCGTGATA TICGCACTAT		-	
10001		CCGCGAGGGA GGCGCTCCCT			
10051		GGCGCGAGCT CCGCGCTCGA			
10101		GACTTTGAGC CTGAAACTCG			
10151	GCGCACACGT CGCGTGTGCA	CCGCCGGCGG	GACCTGGTAA CTGGACCATT	CCGCATACGA GGCGTATGCT	GCAGACGGTG CGTCTGCCAC
10201	AACCAGGAGA TTGGTCCTCT	TTAACTTTCA AATTGAAAGT			
	TGTGGCGCGC ACACCGCGCG				
10301	TAAGCGCGCT ATTCGCGCGA	GGAGCAAAAC CCTCGTTTTG	CCAAATAGCA GGTTTATCGT	AGCCGCTCAT TCGGCGAGTA	GGCGCAGCTG CCGCGTCGAC

Figure 27K

10351	TTCCTTATAG AAGGAATATC	T GCACAG ACGICGTGTC	CAGGGACAAC GTCCCTGTTG	GAGGCATTCA CTCCGTAAGT	GGGATG T CCCTACGGGA
10401			AGGGCCGCTG TCCCGGCGAC		
10451	TCCTGCAGAG AGGACGTCTC	CATAGTGGTG GTATCACCAC	CAGGAGCGCA GTCCTCGCGT	GCTTGAGCCT CGAACTCGGA	GGCTGACAAG CCGACTGTTC
10501	GTGGCCGCCA CACCGGCGGT	TCAACTATTC AGTTGATAAG	CATGCTTAGC GTACGAATCG	CTGGGCAAGT GACCCGTTCA	TTTACGCCCG AAATGCGGGC
10551	CAAGATATAC GTTCTATATG	CATACCCCTT GTATGGGGAA	ACGTTCCCAT TGCAAGGGTA	AGACAAGGAG TCTGTTCCTC	GTAAAGATCG CATTTCTAGC
10601			GCGCTGAAGG CGCGACTTCC		
10651	GACCCGCAAA	TAGCGTTGCT	GCGCATCCAC CGCGTAGGTG	TTCCGGCACT	CGCACTCGGC
10701	CGCCGCGCTC	GAGTCGCTGG		CGTGTCGGAC	STTTCCCGGG
10751	ACCGACCGTG	CCCGTCGCCG	CTATCTCTCC	GGCTCAGGAT	
10801	CCGCGACTGG	ACGCGACCCG	GGGTTCGGCT	GCGCGGGACC	AGGCAGCTGG TCCGTCGACC
10851	CCGGCCTGGA	CCCGACCGCC	ACCGTGGGCG	CGCGCGACCG	AACGTCGGCG TTGCAGCCGC
10901	CGCACCTCCT	TATACTGCTC	CTGCTACTCA	TGCTCGGTCT	GGACGGCGAG CCTGCCGCTC
10951	ATGATTCGCC	ACTACAAAGA	CTAGTCTACT	ACGTTCTGCG	AACGGACCCG TTGCCTGGGC
11001	CGCCACGCCC	GCCGCGACGT	CTCGGTCGGC	AGGCCGGAAT	ACTCCACGGA TGAGGTGCCT
	GCTGACCGCG	GTCCAGTACC	TGGCGTAGTA	CAGCGACTGA	GCGCGCAATC CGCGCGTTAG
11101	GACTGCGCAA	GGCCGTCGTC	GGCGTCCGGT	TGGCCGAGAG	CGCAATTCTG CCCTTAAGAC
11151	CTTCGCCACC	AGGGCCGCGC	GCGTTTGGGG	TGCGTGCTCT	AGGTGCTGGC TCCACGACCG
	CTAGCATTTG	CGCGACCGGC	TTTTGTCCCG	GTAGGCCGGG	GACGAGGCCG CTGCTCCGGC
11251	GCCTGGTCTA CGGACCAGAT	CGACGCGCTG	CTTCAGCGCG GAAGTCGCGC	TGGCTCGTTA ACCGAGCAAT	CAACAGEGGE GTTGTEGEEG

Figure 27L

11301	AACGTGCAGA TTGCACGTCT	CCTGGA GCTTGGACCT	CCGGĆTGGTG GGCCGACCAC	GGGGATGTGC CCCCTACACG	GCGAGG T CGCTCCGGCA
11351	GGCGCAGCGT	GAGCGCGCGC	AGCAGCAGGG	CAACCTGGGC	TCCATGGTTG
	CCGCGTCGCA	CTCGCGCGCG	TCGTCGTCCC	GTTGGACCCG	AGGTACCAAC
11401	CACTAAACGC GTGATTTGCG	CTTCCTGAGT GAAGGACTCA	ACACAGCCCG TGTGTCGGGC	CCAACGTGCC GGTTGCACGG	CCCCCTGTC
11451	GAGGACTACA	CCAACTTTGT	GAGCGCACTG	CGGCTAATGG	TGACTGAGAC
	CTCCTGATGT	GGTTGAAACA	CTCGCGTGAC	GCCGATTACC	ACTGACTCTG
11501	TGGCGTTTCA	CTCCACATGG	TCAGACCCGG	AGACTATTTT TCTGATAAAA	AAGGTCTGGT
11551	CATCTGTTCC	GGACGTCTGG	CATTTGGACT	GCCAGGCTTT CGGTCCGAAA	GTTTTTGAAC
11601	GTCCCCGACA	CCCCCCACGC	CCGAGGGTGT	GGCGACCGCG CCGCTGGCGC	GCTGGCACAG
11651	TAGCTTGCTG	ACGCCCAACT	CGCGCCTGTT	GCTGCTGCTA	ATAGCGCCCT
	ATCGAACGAC	TGCGGGTTGA	GCGCGGACAA	CGACGACGAT	TATCGCGGGA
11701	TCACGGACAG	TGGCAGCGTG	TCCCGGGACA	CATACCTAGG	TCACTTGCTG
	AGTGCCTGTC	ACCGTCGCAC	AGGGCCCTGT	GTATGGATCC	AGTGAACGAC
11751	ACACTGTACC	GCGAGGCCAT	AGGTCAGGCG	CATGTGGACG	AGCATACTTT
	TGTGACATGG	CGCTCCGGTA	TCCAGTCCGC	GTACACCTGC	TCGTATGAAA
11801	CCAGGAGATT	ACAAGTGTCA	GCCGCGCGCT	GGGGCAGGAG	GACACGGGCA
	GGTCCTCTAA	TGTTCACAGT	CGGCGCGCGA	CCCCGTCCTC	CTGTGCCCGT
11851	GCCTGGAGGC	AACCCTAAAC	TACCTGCTGA	CCAACCGGCG	GCAGAAGATC
	CGGACCTCCG	TTGGGATTTG	ATGGACGACT	GGTTGGCCGC	CGTCTTCTAG
11901	CCCTCGTTGC	ACAGTTTAAA	CAGCGAGGAG	GAGCGCATTT	TGCGCTACGT
	GGGAGCAACG	TGTCAAATTT	GTCGCTCCTC	CTCGCGTAAA	ACGCGATGCA
11951	GCAGCAGAGC	GTGAGCCTTA	ACCTGATGCG	CGACGGGGTA	ACGCCCAGCG
	CGTCGTCTCG	CACTCGGAAT	TGGACTACGC	GCTGCCCCAT	TGCGGGTCGC
12001	ACCGCGACCT	GTACTGGCGC	GCGTTGTACC	AACCGGGCAT TTGGCCCGTA	CATACGGAGT
12051	AACCGGCCGT TTGGCCGGCA	TTATCAACCG AATAGTTGGC	CCTAATGGAC GGATTACCTG	TACTTGCATC ATGAACGTAG	CGCGCCGCC
12101	CGTGAACCCC	GAGTATTTCA	CCAATGCCAT	CTTGAACCCG	CACTGGCTAC
	GCACTTGGGG	CTCATAAAGT	GGTTACGGTA	GAACTTGGGC	GTGACCGATG
12151	CGCCCCCTGG	TTTCTACACC	GGGGGATTCG	AGGTGCCCGA	GGGTAACGAT
	GCGGGGGACC	AAAGATGTGG	CCCCTAAGC	TCCACGGGCT	CCCATTGCTA
12201	GGATTCCTCT	GGGACGACAT	AGACGACAGC	GTGTTTTCCC	CGCAACCGCA
	CCTAAGGAGA	CCCTGCTGTA	TCTGCTGTCG	CACAAAAGGG	GCGTTGGCGT

Figure 27 M

12251	GACCCTGCTA CTGGGACGAT	GTTGCAAC CLAACGTTG	AGCGCGAGCA TCGCGCTCGT	GGCAGAGGCG CCGTCTCCGC	CCCCTC
12301				CCGATCTAGG GGCTAGATCC	
12351				AGCTTGATAG TCGAACTATC	
12401				GGGCGAGGAG CCCGCTCCTC	
12451				AAAACCTGCC TTTTGGACGG	
12501				AAGATGAGTA TTCTACTCAT	
12551				GGGCGCGGGC	
12601				TGTGGGAGGA ACACCCTCCT	
12651				GGGAGTGGCA CCCTCACCGT	
12701				TTAAAAAAAA TTTTTTTTAA	
12751				GCACCGAGCG CGTGGCTCGC	
12801				ATGTATGAGG TACATACTCC	
12851				GCCAGTGGCG CGGTCACCGC	GCGGCGACC
12901				CGTTTGTGCC GCAAACACGG	
12951	CTGCGGCCTA GACGCCGGAT				
13001					TCAACGGATG AGTTGCCTAC
13051	TGGCATCCCT ACCGTAGGGA				
13101	ATTCAAAACA TAAGTTTTGT				AGACCATCAA TCTGGTAGTT
13151					ATCCTGCATA TAGGACGTAT



13201	CCAACATGCC GGTTGTACGG	AZ TGTGAAC T CACTTG	GAGTTCATGT CTCAAGTACA	TTACCAATAA AATGGTTATT	CAAATT C
13251	CGGGTGATGG GCCCACTACC	TGTCGCGCTT ACAGCGCGAA	GCCTACTAAG CGGATGATTC	GACAATCAGG CTGTTAGTCC	TGGAGCTGAA ACCTCGACTT
13301	ATACGAGTGG TATGCTCACC	GTGGAGTTCA CACCTCAAGT	CGCTGCCCGA GCGACGGGCT	GGGCAACTAC CCCGTTGATG	TCCGAGACCA AGGCTCTGGT
13351				TGGAGCACTA ACCTCGTGAT	
13401	CCGTCTGTCT	TGCCCCAAGA	CCTTTCGCTG	ATCGGGGTAA TAGCCCCATT	TCAAACTGTG
13451	GGCGTTGAAG	TCTGACCCCA	AACTGGGGCA	CACTGGTCTT GTGACCAGAA	CAGTACGGAC
13501	CCCATATATG	TTTGCTTCGG	AAGGTAGGTC	ACATCATTTT TGTAGTAAAA	CGACGGTCCT
13551	ACGCCCCACC	TGAAGTGGGT	GTCGGCGGAC	AGCAACTTGT TCGTTGAACA	ACCCGTAGGC
13601	GTTCGCCGTT	GGGAAGGTCC	TCCCGAAATC	GATCACCTAC CTAGTGGATG	CTACTAGACC
13651	TCCCACCATT	GTAAGGGCGT	GACAACCTAC	TGGACGCCTA ACCTGCGGAT	GGTCCGCTCG
13701	AACTTTCTAC	TGTGGCTTGT	CCCGCCCCCA	GGCGCAGGCG CCGCGTCCGC	CGTCGTTGTC
13751	GTCACCGTCG	CCGCGCCTTC	TCTTGAGGTT	CGCGGCAGCC	CGCCGTTACG
13801	TCGGCCACCT	CCTGTACTTG	CTAGTACGGT	TTCGCGGCGA AAGCGCCGCT	GTGGAAACGG
13851	TGTGCCCGAC	TCCTCTTCGC	GCGACTCCGG	GAAGCAGCGG CTTCGTCGCC	GGCTTCGACG
13901	CGCCCCCGCT	GCGCAACCCG CGCGTTGGGC	AGGTCGAGAA TCCAGCTCTT	GCCTCAGAAG CGGAGTCTTC	AAACCGGTGA TTTGGCCACT
		CTGTCTCCTG	TCGTTCTTTG	CGTCAATGTT	GGATTATTCG
		GGAAGTGGGT	CATGGCGTCG	ACCATGGAAC	GTATGTTGAT
		GTCTGGCCTT	AGGCGAGTAC	CTGGGACGAA	ACGTGAGGAC
14101	ACGTAACCTG TGCATTGGAC	CGGCTCGGAG GCCGAGCCTC	CAGGTCTACT GTCCAGATGA	GGTCGTTGCC CCAGCAACGG	AGACATGATG TCTGTACTAC

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14151	CAAGACCCCG	TTTCCG	CTCCACGCGC	CAGATCAGCA	ACTTTC( T
	GTTCTGGGGC	ACTGGAAGGC	GAGGTGCGCG	GTCTAGTCGT	TGAAAGGCCA
14201	GCTGGGGGCC	CAGCTGTTGC	CCGTGCACTC	CAAGAGCTTC	TACAACGACC
	CCACCGGGG	CTCGACAACG	GGCACGTGAG	GTTCTCGAAG	ATGTTGCTGG
14251	AGGCCGTCTA	CTCCCAACTC	ATCCGCCAGT	TTACCTCTCT	GACCCACGTG
	TCCGGCAGAT	CAGGCTTGAG	TAGGCGCTCA	AATGGAGAGA	CTGGGTGCAC
14301		TTCCCGAGAA AAGGGCTCTT			
14351	CATCACCACC	GTCAGTGAAA	ACGTTCCTGC	TCTCACAGAT	CACGGGACGC
	GTAGTGGTGG	CAGTCACTTT	TGCAAGGACG	AGAGTGTCTA	GTGCCCTGCG
14401	TACCGCTGCG	CAACAGCATC	GCAGGAGTCC	ACCGACTGAC	CATTACTGAC
	ATGGCGACGC	GTTGTCGTAG	CCTCCTCAGG	TCGCTCACTG	GTAATGACTG
14451	GCCAGACGCC	GCACCTGCCC	CTACGTTTAC	AAGGCCCTGG	GCATAGTCTC
	CGGTCTGCGG	CGTGGACGGG	GATGCAAATG	TTCCGGGACC	CGTATCAGAG
14501	GCCGCGCGTC	CTATCGAGCC GATAGCTCGG	GCACTTTTTG CGTGAAAAAC	AGCAAGCATG TCGTTCGTAC	TCCATCCTTA AGGTAGGAAT
14551	TATCGCCCAG ATAGCGGGTC	CAATAACACA GTTATTGTGT	GGCTGGGGCC CCGACCCCGG	TGCGCTTCCC	AAGCAAGATG TTCGTTCTAC
14601	TTTGGCGGGG	CCAAGAAGCG	CTCCGACCAA	CACCCAGTGC	GCGTGCGCGG
	AAACCGCCCC	GGTTCTTCGC	GAGGCTGGTT	GTGGGTCACG	CGCACGCGCC
14651	GCACTACCGC	GCGCCCTGGG	GCGCGCACAA	ACGCGGCCGC	ACTGGGCGCA
	CGTGATGGCG	CGCGGGACCC	CGCGCGTGTT	TGCGCCGGCG	TGACCCGCGT
14701	CCACCGTCGA	TGACGCCATC	GACGCGGTGG	TGCAGGAGGC	GCGCAACTAC
	GGTGGCAGCT	ACTGCGGTAG	CTGCGCCACC	ACCTCCTCCG	CGCGTTGATG
14751	ACGCCCACGC	CGCCACCAGT	GTCCACAGTG	GACGCGGCCA	TTCAGACCGT
	TGCGGGTGCG	GCGGTGGTCA	CAGGTGTCAC	CTGCGCCGGT	AAGTCTGGCA
14801	GGTGCGCGGA CCACGCGCCT	GCCCGGCGCT CGGGCCGCGA	ATGCTAAAAT TACGATTITA	GAAGAGACGG CTTCTCTGCC	CGGAGGCGCGC
14851	TAGCACGTCG	CCACCGCCGC	CGACCCGGCA	CTGCCGCCCA	ACGCGCGCGC
	ATCGTGCAGC	GCTGGCGGCG	GCTGGGCCGT	GACGGCGGGT	TGCGCGCCGC
14901	GCGGCCCTGC CGCCGGGACG	TTAACCGCGC AATTGGCGCG	ACGTCGCACC TGCAGCGTGG	GGCCGACGGG	CGGCCATGCG
14951	CCCCCTCGA	AGGCTGGCCG	CGGGTATTGT	CACTGTGCCC	CCCAGGTCCA
	CCGGCGAGCT	TCCGACCGGC	GCCCATAACA	GTGACACGGG	GGGTCCAGGT
15001	GGCGACGAGC CCGCTGCTCG	GGCCGCCGCA TDCQQCCGCCT	GCAGCCGCGG	CCATTAGTGC GGTAATCACG	TATGACTCAG ATACTGAGTC
15051	GGTCGCAGGG CCAGCGTCCC	GCAACGTGTA CGTTGCACAT	TTGGGTGCGC AACCCACGCG	GACTCGGTTA CTGAGCCAAT	CGCCGGACGC

Figure 27P

15101	CGTGCCCGTG GCACGGGCAC	600000	CCCCGCGCAA GGGGCGCGTT	CTAGATTGCA GATCTAACGT	TCTTTTT.A
15151			ATGTATCCAG TACATAGGTC		
15201			CAAAGAAGAG GTTTCTTCTC		
15251			AGAAGGAAGA TCTTCCTTCT		
15301			AAAAAGAAAG TTTTTCTTTC		
15351	GACGAGGTGG CTGCTCCACC	AACTGCTGCA TTGACGACGT	CGCTACCGCG GCGATGGCGC	CCCAGGCGAC GGGTCCGCTG	GGGTACAGTG CCCATGTCAC
15401	CTTTCCAGCT	GCGCATTTTG	GTGTTTTGCG CACAAAACGC	TGGGCCGTGG	TEGCATCAGA
15451			ACCCGCACCT TGGGCGTGGA		
15501			GCTTGAGCAG CGAACTCGTC		
15551	GTTTGCCTAC CAAACGGATG	GGAAAGCGGC CCTTTCGCCG	ATAAGGACAT TATTCCTGTA	GCTGGCGTTG CGACCGCAAC	CCGCTGGACG GGCGACCTGC
15601	AGGGCAACCC TCCCGTTGGG	AACACCTAGC TTGTGGATCG	CTAAAGCCCG GATTTCGGGC	TAACACTGCA ATTGTGACGT	GCAGGTGCTG CGTCCACGAC
15651	CCCGCGCTTG GGGCGCGAAC	CACCETCCGA GTGGCAGGCT	AGAAAAGCGC TCTTTTCGCG	GGCCTAAAGC CCGGATTTCG	GCGAGTCTGG CGCTCAGACC
15701			AGCTGATGGT TCGACTACCA		
15751			ACCGTGGAAC TCGCACCTTG		
15801			GGTGGCGCCG CCACCGCGGC		
15851	GGACGTTCAG CCTGCAAGTC	ATACCCACTA TATGGGTGAT	CCAGTAGCAC GGTCATCGTG	CAGTATTGCC GTCATAACGG	ACCGCCACAG TGGCGGTGTC
15901	AGGGCATGGA TCCCGTACCT	GACACAAACG CTGTGTTTGC	TCCCCGCTTG AGGGGCCAAC	CCTCAGCGGT GGAGTCGCCA	CCCCGATGCC CCGCCTACGG
15951	GCGCTGCAGG CGCCACGTCC	CGGTCGCTGC GCCAGCGACG	GCCCGCGTCC CCGGCGCAGG	AAGACCTCTA TTCTGGAGAT	CGGAGGTGCA GCCTCCACGT
16001			GCGTTTCAGC CGCAAAGTCG		

Figure 270

16051				TGCCCTATT ACGGGATGTA
16101				ACCGCCCCAG TGGCGGGGTC
16151		ACTACCCGAC TGATGGGCTG		6666666666 666666666666666666666666666
16201				CAGGGTGGCT GTCCCACCGA
16251		GCAGGACCCT CGTCCTGGGA		
16301				GCCCTCACCT CGGGAGTGGA
16351		TTTCCCGGTG AAAGGGCCAC		
16401		CCGGCCACGG GGCCGGTGCC		GTGCGCACCA CACGCGTGGT
16451		CGCGCGTCGC GCGCGCAGCG		
16501		ACTGATCGCC TGACTAGCGG		
16551		TGCAGGCGCA ACGTCCGCGT		
16601		AATAAAAAGT TTATTTTCA		
16651		GAATGGAAGA CTTACCTTCT		
16701		CCGTTCATGG GGCAAGTACC		
16751		CGCCTTCAGC GCGGAAGTCG		
16801	TTCGGTTCCA AAGCCAAGGT	CCGTTAAGAA GGCAATTCTT		
16851	AGGCCAGATG TCCGGTCTAC			
16901	TGGTAGATGG ACCATCTACC			
16951	CAGGCAGTGC GTCCGTCACG			

Figure 27R

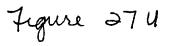
17001					GGGCGTY G CCCGCACCGC
17051				CTCTGGTGAC GAGACCACTG	
17101				CAAGGCCTGC GTTCCGGACG	CCACCACCCG GGTGGTGGGC
17151	TCCCATCGCG AGGGTAGCGC			GGGCCAGCAC CCCGGTCGTG	
17201				AGCAGAAACC TCGTCTTTGG	TGTGCTGCCA ACACGACGGT
17251				AGCCGCGCGT TCGGCGCGCA	
17301				CGTAGCCAGT GCATCGGTCA	
17351	AAAGCACACT TTTCGTGTGA			GGGTGCAATC CCCACGTTAG	
17401				ATGTGTGTCA TACACACAGT	
17451		•••••		eccececece ccecececcc	
17501				TCTTACATGC AGAATGTACG	
17551				GCTGGTGCAG CGACCACGTC	
17601				AGTTTAGAAA TCAAATCTTT	
17651				TCCCAGCGTT AGGGTCGCAA	
17701				GTACTCGTAC CATGAGCATG	
17751	TCACCCTAGC AGTGGGATCG			TGGACATGGC ACCTGTACCG	
	TTTGACATCC AAACTGTAGG				
17851	TGGCACTGCC ACCGTGACGG				
17901	AATGGGATGA TTACCCTACT	AGCTGCTACT TCGACGATGA	GCTCTTGAAA CGAGAACTTT	TAAACCTAGA ATTTGGATCT	AGAAGAGGAC TCTTCTCCTG

Figure 275

17951		A CGAAGT TTCTGCTTCA			
18001	CGTATTTGGG GCATAAACCC	CAGGCGCCTT GTCCGCGGAA	ATTCTGGTAT TAAGACCATA	AAATATTACA TITATAATGT	AAGGAGGGTA TTCCTCCCAT
18051		TGTCGAAGGT ACAGCTTCCA			
18101		CTCAAATAGG GAGTTTATCC			
18151		GGGAGAGTCC CCCTCTCAGG			
18201		TGCAAAACCC ACGTTTTGGG			
18251		AAAATGGAAA TTTTACCTTT			
18301		GAGGCAGCCG CTCCGTCGGC			
18351		CAGTGAAGAT GTCACTTCTA			
18401	-	CCACTATTAA GGTGATAATT			
18451		CCCAACAGGC GGGTTGTCCG			
18501		GTATTACAAC CATAATGTTG			
18551		AGTTGAATGC TCAACTTACG			
18601		CAGCTTTTGC GTCGAAAACG			
18651		GAATCAGGCT CTTAGTCCGA			
18701	ATTGAAAATC TAACTTTTAG	ATGGAACTGA TACCTTGACT			
18751	GGGAGGTGTG CCCTCCACAC	ATTAATACAG TAAITATGTC			
18801	GTCAGGAAAA CAGTCCTTTT	TGGATGGGAA ACCTACCCTT			
	GAAATAAGAG CTTTATTCTC				

Figure 27T

18901			ACTCCAACAT TGAGGTTGTA		
18951			AACGTAAAAA TTGCATTTTT		
19001	TACGACTACA ATGCTGATGT		AGTGGTGGCT TCACCACCGA		
19051			GGTCCCTTGA CCAGGGAACT		
19101	-		GCTGGCCTGC CGACCGGACG		
19151			CTTCCACATC GAAGGTGTAG		
19201			TCCTGCCGGG AGGACGGCCC		
19251			ATGGTTCTGC TACCAAGACG		
19301			CATTAAGTTT GTAATTCAAA		
19351			ACAACACCGC TGTTGTGCCG		
19401			CAGTCCTTTA GTCAGGAAAT		
19451			CGCCAACGCT GCGGTTGCGA		
19501			CTTTCCGCGG GAAAGGCGCC		
19551			CTGGGCTCGG GACCCGAGCC		
19601			CCTAGATGGA GGATCTACCT		
19651	CTTTAAGAAG GAAATTCTTC	GTGGCCATTA CACCGGTAAT	CCTTTGACTC GGAAACTGAG	TTCTGTCAGC AAGACAGTCG	TGGCCTGGCA ACCGGACCGT
19701	ATGACCGCCT TACTGGCGGA	GCTTACCCCC CGAATGGGGG	AACGAGTTTG TTGCTCAAAC	AAATTAAGCG TTTAATTCGC	CTCAGTTGAC GAGTCAACTG
19751	GGGGAGGGTT CCCCTCCCAA	ACAACGTTGC TGTTGCAACG	CCAGTGTAAC GGTCACATTG	ATGACCAAAG TÄCTGGTTTC	ACTGGTTCCT TGACCAAGGA
19801	GGTACAAATG CCATGTTTAC	CTAGCTAACT GATCGATTGA	ATAACATTGG TATTGTAACC	CTACCAGGGC GATGGTCCCG	TTCTATATCC AAGATATAGG



19851	CAGAGAGCTA GTCTCTCGAT			CTTCCA(CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
19901		AGGTGGTGGA TCCACCACCT		ACCAACAGGT TGGTTGTCCA
19951	GGGCATCCTA CCCGTAGGAT	CACCAACACA GTGGTTGTGT	 	
20001		CGAAGGACAG GCTTCCTGTC		CTATCCGCTT GATAGGCGAA
20051	ATAGGCAAGA TATCCGTTCT	CCGCAGTTGA GGCGTCAACT		
20101		TGGCGCATCC ACCGCGTAGG		
20151	CACTCACAGA GTGAGTGTCT	CCTGGGCCAA GGACCCGGTT		
20201		CTTTTGAGGT GAAAACTCCA		CCCTTCTTTA GGGAAGAAAT
20251		GAAGTCTTTG CTTCAGAAAC		
20301		AACCGTGTAC TTGGCACATG		
20351		GAAGCAAGCA CTTCGTTCGT	 	
20401		AACTGAAAGC TTGACTTTCG	 	GTGGGCCATA CACCCGGTAT
20451		ACCTATGACA TGGATACTGT	 	
20501		CGCCATAGTC GCGGTATCAG	 	TGGGGGCGTA ACCCCCGCAT
20551				GCTACCTCTT CGATGGAGAA
20601	TGAGCCCTTT ACTCGGGAAA			TACCAGTTTG ATGGTCAAAC
20651	AGTACGAGTC TCATGCTCAG			CCCCGACCGC GGGGCTGGCG
20701	TGTATAACGC ACATATTGCG			CCAACTCGGC GGTTGAGCCG
20751	CGCCTGTGGA GCGGACACCT			GCCAACTGGC CGGTTGACCG

Figure 27V

20801	CCCAAACTCC GGGTTTGAGG	CATCAC GTACCTAGTG	AACCCCACCA TTGGGGTGGT	TGAACCTTAT ACTTGGAATA	TACCGG AT
20851			TCCCCAGGTA AGGGGTCCAT		
20901			TCCTGGAGCG AGGACCTCGC		
20951			AGCGCCACTT TCGCGGTGAA		
21001			AGACACTTTC TCTGTGAAAG		
21051	AAACATGTGA	GAGCCCACTA	TATTTACCCC ATAAATGGGG	GTGGGAACGG	CAGACGCGGC
21101	AAATTTTTAG	TTTCCCCAAG	TGCCGCGCAT ACGGCGCGTA	GCGATACGCG	GTGACCGTCC
21151	CTGTGCAACG	CTATGACCAC	TTTAGTGCTC AAATCACGAG	GTGAATTTGA	GTCCGTGTTG
21201	GTAGGCGCCG	TCGAGCCACT	AGTTTTCACT TCAAAAGTGA	GGTGTCCGAC	GCGTGGTAGT
21251	GGTTGCGCAA	ATCGTCCAGC	GGCGCCGATA CCGCGGCTAT	AGAACTTCAG	CGTCAACCCC
21301	GGAGGCGGGA	CGCGCGCGCT	GTTGCGATAC CAACGCTATG	TGTCCCAACG	TCGTGACCTT
21351	GTGATAGTCG	CGGCCCACCA	GCACGCTGGC CGTGCGACCG	GTCGTGCGAG	AACAGCCTCT
21401	AGTCTAGGCG	CAGGTCCAGG	TCCGCGTTGC AGGCGCAACG	AGTCCCGCTT	GCCTCAGTTG
21451	AAACCATCGA	CGGAAGGGTT	AAAGGGCGCG TTTCCCGCGC	ACGGGTCCGA	AACTCAACGT
21501	GAGCGTGGCA	TCACCGTAGT	AAAGGTGACC TTTCCACTGG	CACGGGCCAG	ACCCGCAATC
•		GACGTATTTT	CGGAACTAGA	CGAATTTTCG	GTGGACTCGG
		GTCTCTTCTT	GTACGGCGTT	CTGAACGGCC	TTTTGACTAA
	CCGGCCTGTC	CGGCGCAGCA	CETECGICGT	GGAACGCAGC	GTGTTGGAGA CACAACCTCT
21701	TCTGCACCAC AGACGTGGTG	ATTTCGGCCC TAAAGCCGGG	CACCGGTTCT GTGGCCAAGA	TCACGATCTT AGTGCTAGAA	GGCCTTGCTA CCGGAACGAT

7, gure 27 W

21751	GACTGCTCCT CTGACGAGGA		CTGCCCGTTT GACGGGCAAA		
21801			TCATAATGCT AGTATTACGA		
21851			CGGTGCAGCC GCCACGTCGG		
21901			CTCTGCAAAC GAGACGTTTG		
21951			CAAAGGTCTT GTTTCCAGAA		
22001			TTCAGCCAGG AAGTCGGTCC		
22051	•••••		TAGTTTGAAG ATCAAACTTC		
22101			CGCGCGCGCGC		
22151			CTCAGCGGGT GAGTCGCCCA		
22201			CTCTTCCTCT GAGAAGGAGA		
22251			GCCGCCGCAC CGCCGCGTG		
22301			GGGTTGCTGA CCCAACGACT		
22351			GCTGTCCACG CGACAGGTGC		GTGATGGCGG CACTACCGCC
22401			GGCGCTTCTT CCGCGAAGAA		
22451					GCGCGCCACC
22501	AGCGCGTCTT TCGCGCAGAA	GTGATGAGTC CACTACTCAG	TTCCTCGTCC AAGGAGCAGG	TCGGACTCGA AGCCTGAGCT	TACGCCGCCT ATGCGGCGGA
22551	CATCCGCTTT GTAGGCGAAA				GGGGACGGGG CCCCTGCCCC
	ACGACACGTC TGCTGTGCAG				
22651	TCGGGGGTGG AGCCCCCACC				TTTCCTTCTC AAAGGAAGAG

Figure 27X

22701	CTATACCCAG GATATCCGTC	A AGATCA TTTTTCTAGT	TGGAGTCAGT ACCTCAGTCA	CGAGAAGAAG GCTCTTCTTC	GACAGCO A CTGTCGGATT
22751		TGAGTTCGCC ACTCAAGCGG			
22801		TCCCCGTCGA AGGGGCAGCT			
22851		GACCCAGGTT CTGGGTCCAA			
22901		GGATAAAAAG CCTATTTTTC			
22951		GGCGGGGGA CCGCCCCCT			
23001		CTGTTGAAGC GACAACTTCG			
23051		AGAGCGCAGC TCTCGCGTCG			
23101		AACGCCACCT TTGCGGTGGA			
23151		ACATGCGAGC TGTACGCTCG			
23201		AGAGGTGCTT TCTCCACGAA			
23251		TATCCTGCCG ATAGGACGGC			
23301		CAGGGCGCTG GTCCCGCGAC			
23351		CTTTGAGGGT GAAACTCCCA			
23401		AGGAAAACAG TCCTTTTGTC			
23451	GGAACTCGAG CCTTGAGCTC	GGTGACAACG CCACTGTTGC			
23501	AGGTCACCCA TCCAGTGGGT	CTTTGCCTAC GAAACGGATG			
23551	AGCACAGTCA TCGTGTCAGT	TGAGTGAGCT ACTCACTCGA			
23601	GGATGCAAAT CCTACGTTTA	TTGCAAGAAC AACGTTCTTG			

Figure 27 Y

23651	ACGAGCAGCT TGCTCGTCGA	A CGCTGG TCGCGCGACC			
23701		AACTAATGAT TTGATTACTA			TGGAGCTTGA ACCTCGAACT
23751		CGCTTCTTTG GCCAAGAAAC			
23801		CTACACCTTT GATGTGGAAA			
23851		TGGAGCTCTG ACCTCGAGAC			
23901		CTTGGGCAAA GAACCCGTTT			
23951		CTACGTCCGC GATGCAGGCG			
24001		CCATGGGCGT GGTACCCGCA			
24051		CAGAAACTGC GTCTTTGACG			
24101		GCGCTCCGTG CGCGAGGCAC			
24151		TTAAAACCCT AATTTTGGGA			
24201	AAGCATGTTG	CAGAACTTTA GTCTTGAAAT	GGAACTTTAT	CCTAGAGCGC	TCAGGAATCT
24251	TGCCCGCCAC	CTGCTGTGCA GACGACACGT	CTTCCTAGCG	ACTTTGTGCC	CATTAAGTAC
24301	CGCGAATGCC	CTCCGCCGCT GAGGCGGCGA	TTGGGGCCAC	TGCTACCTTC	TGCAGCTAGC
24351	CAACTACCTT	GCCTACCACT CGGATGGTGA	CTGACATAAT	GGAAGACGTG	AGCGGTGACG
24401	GTCTACTGGA CAGATGACCT	GTGTCACTGT	CGCTGCAACC	TATGCACCCC	GCACCGCTCC
24451	CTGGTTTGCA GACCAAACGT	ATTCGCAGCT	GCTTAACGAA	agtcaaatta	TCGCTACCTT
	TGAGCTGCAG ACTCGACGTC	GGTCCCTCGC	CTGACGAAAA	GTCCGCGGCT	CCGGGGTTGA
	AACTCACTCC TTGAGTGAGG	GGGGCTGTGG	ACGICGGCIT	ACCTTCGCAA	ATTTGTACCT

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				•	
24601	GAGGACTACC	ACCCACGA	GATTAGGTTC	TACGAAGACC	AATCCCGGCC
		TGCGGGTGCT			
	C.CC. 0/1.00	.000001001			,
24651	CCCTAATCCC	GAGCTTACCG	CCTCCCCCCATC	macccaccc	CACAMMOMMO
24031	- +				
	CGGATTACGC	CTCGAATGGC	GGACGCAGTA	ATGGGTCCCG	GTGTAAGAAC
24701	••••	AGCCATCAAC			
	CGGTTAACGT	TCGGTAGTTG	TTTCGGGCGG	TTCTCAAAGA	CGATGCTTTC
24751	GGACGGGGGG	TTTACTTGGA	CCCCCAGTCC	GGCGAGGAGC	TCAACCCAAT
	CCTGCCCCCC	AAATGAACCT	GGGGGTCAGG	CCGCTCCTCG	AGTTGGGTTA
24801	ccccccccc	CCGCAGCCCT	ATCAGCAGCA	GCCGCGGGCC	CTTGCTTCCC
		GGCGTCGGGA			
	0000000000	GGCGTCGGGA	INGICOICGI	COOCGEECCO	Gradu de la companya
24051	> 00 > 00 C > 0	CCAAAAAGAA	CCMCCNCCMC	******	CCACCCACCA
24851	• • • • • • • • • • • • • • • • • • • •				
	TCCTACCGTG	GGTTTTTCTT	CGACGTCGAC	فالافافاتافافاتافافا	GGTGCCTGCT
24901		TGGGACAGTC			
	CCTCCTTATG	ACCCTGTCAG	TCCGTCTCCT	CCAAAACCTG	CTCCTCCTCC
24951	AGGACATGAT	GGAAGACTGG	GAGAGCCTAG	ACGAGGAAGC	TTCCGAGGTC
	TCCTGTACTA	CCTTCTGACC	CTCTCGGATC	TGCTCCTTCG	AAGGCTCCAG
25001	GAAGAGGTGT	CAGACGAAAC	ACCGTCACCC	TCGGTCGCAT	TCCCCTCGCC
22001		GTCTGCTTTG			
	CITCICCACA	9101001110	1000000	nocchocoz	
25053	0000000000	AAATCGGCAA	CCCCDDCCAC	CARCCCRACA	3 CCMCCCCMC
25051					-
	CCGCGGGGTC	TTTAGCCGTT	GGCCAAGGTC	GTACCGATGT	TGSAGGCGAG
25101		GCCGGCACTG			
	GAGTCCGCGG	CGGCCGTGAC	GGGCAAGCGG	CTGGGTTGGC	ATCTACCCTG
			*	-	
25151	ACCACTGGAA	CCAGGGCCGG	TAAGTCCAAG	CAGCCGCCGC	CGTTAGCCCA
	TGGTGACCTT	GGTCCCGGCC	ATTCAGGTTC	GTCGGCGGCG	GCAATCGGGT
25201	AGAGCAACAA	CAGCGCCAAG	GCTACCGCTC	ATGGCGCGGG	CACAAGAACG
		GTCGCGGTTC			
	10.0011011	510000110			
25251	CCMMACMMCC	TTGCTTGCAA	CACINCIPOCOCO	CCNNCNTCTC	CTTCCCCCC
23231	••	AACGAACGTT			
	GGTATCAACG	AACGAACG11	CYGACACCCC	CGTTGTAGAG	GAAGCGGGGG
			00000000	mmnoooooo	1 01 moomoo-
25301		TCTACCATCA			
	GCGAAAGAAG	AGATGGTAGT	GCCGCACCGG	AAGGGGGCAT	TGTAGGACGT
25351	TTACTACCGT				
•	AATGATGGCA	GTAGAGATGT	CGGGTATGAC	GTGGCCGCCG	TCGCCGTCGT
25401	ACAGCAGCGG	CCACACAGAA	GCAAAGGCGA	CCGGATAGCA	AGACTCTGAC
		GGTGTGTCTT			
					<del>-</del>
25/51	AAAGCCCAAG	アアアかんりしかいから	רנינרניבר זיבר	<b>JCC JCC JCC J</b>	CCACCCCTCC
2343T		TTTAGGTGTC			
	1110000110	TINGOIGIC	5	1001001001	CLICGCGACG
			ADS DACS		\\\\
25501	GTCTGGCGCC				
	CAGACCGCGG	CTTCCTTCCC	CATAGCTGGG	CECTCGAATC	TTTGTCCTAA

Figure 27 AA

25551	TTTCCCACTC	TO TGCTAT	ATTTCAACAG	AGCAGGGGCC	AAGAACATA
	AAAGGGTGAG	ACATACGATA	TAAAGTTGTC	TCGTCCCGG	TTCTTGTTCT
25601			CTCTGCGATC GAGACGCTAG		
25651	ATCACAAAAG	CGAAGATCAG	CTTCGGCGCA	CGCTGGAAGA	CGCGGAGGCT
	TAGTGTTTTC	GCTTCTAGTC	GAAGCCGCGT	GCGACCTTCT	GCGCCTCCGA
25701			GCTGACTCTT CGACTGAGAA		
25751			AACTACGTCA TTGATGCAGT		
25801	CGCCAGCACC	TGTTGTCAGC	GCCATTATGA	GCAAGGAAAT	TCCCACGCCC
	GCGGTCGTGG	ACAAÇAGTCG	CGGTAATACT	CGTTCCTTTA	AGGGTGCGGG
25851	TACATGTGGA	GTTACCAGCC	ACAAATGGGA	CTTGCGGCTG	GAGCTGCCCA
	ATGTACACCT	CAATGGTCGG	TGTTTACCCT	GAACGCCGAC	CTCGACGGGT
25901	AGACTACTCA	ACCCGAATAA	ACTACATGAG	CGCGGGACCC	CACATGATAT
	TCTGATGAGT	TGGGCTTATT	TGATGTACTC	GCGCCCTGGG	GTGTACTATA
25951	CCCGGGTCAA	CGGAATACGC	GCCCACCGAA	ACCGAATTCT	CCTGGAACAG
	GGGCCCAGTT	GCCTTATGCG	CGGGTGGCTT	TGGCTTAAGA	GGACCTTGTC
26001	GCGGCTATTA	CCACCACACC	TCGTAATAAC	CTTAATCCCC	GTAGTTGGCC
	CGCCGATAAT	GGTGGTGTGG	AGCATTATTG	GAATTAGGGG	CATCAACCGG
26051	CGCTGCCCTG	GTGTACCAGG	AAAGTCCCGC	TCCCACCACT	GTGGTACTTC
	GCGACGGGAC	CACATGGTCC	TTTCAGGGCG	AGGGTGGTGA	CACCATGAAG
26101	CCAGAGACGC	CCAGGCCGAA	GTTCAGATGA	CTAACTCAGG	GGCGCAGCTT
	GGTCTCTGCG	GGTCCGGCTT	CAAGTCTACT	GATTGAGTCC	CCGCGTCGAA
26151	GCGGGCGGCT	TTCGTCACAG	GGTGCGGTCG	CCCGGGCAGG	GTATAACTCA
	CGCCCGCCGA	AAGCAGTGTC	CCACGCCAGC	GGGCCCGTCC	CATATTGAGT
26201	CCTGACAATC	AGAGGGCGAG	GTATTCAGCT	CAACGACGAG	TCGGTGAGCT
	GGACTGTTAG	TCTCCCGCTC	CATAAGTCGA	GTTGCTGCTC	AGCCACTCGA
26251	CCTCGCTTGG GGAGCGAACC	TCTCCGTCCG AGAGGCAGGC	GACGGGACAT CTGCCCTGTA	TŢCAGATCGG AAGTCTAGCC	CGCCCCCCCC
26301	CGCTCTTCAT	TCACGCCTCG	TCAGGCAATC	CTAACTCTGC	AGACCTCGTC
	GCGAGAAGTA	AGTGCGGAGC	AGTCCGTTAG	GATTGAGACG	TCTGGAGCAG
26351	CTCTGAGCCG	CGCTCTGGAG	GCATTGGAAC	TCTGCAATTT	ATTGAGGAGT
	GAGACTCGGC	GCGAGACCTC	CGTAACCTTG	AGACGTTAAA	TAACTCCTCA
26401	TTGTGCCATC AACACGGTAG	GGTCTACTTT CCAGATGAAA	AACCCCTTCT TTGGGGAAGA	CCCTGGAGG	CGGCCACTAT GCCGGTGATA
26451	CCGGATCAAT GGCCTAGTTA	TTATTCCTAA AATAAGGATT	CTTTGACGCG GAAACTGCGC	GTAAAGGACT CATTTCCTGA	CGGCGGACGG

Figure 27 AB

26501	CTACGACTGA	ATAAGTG	GAGAGGCAGA	GCAACTGCGC	CTGAAA
	GATGCTGACT	TACAATTCAC	CTCTCCGTCT	CGTTGACGCG	GACTTTGTGG
26551	TGGTCCACTG	TCGCCGCCAC	AAGTGCTTTG	CCCGCGACTC	CGCTGAGTTT
•	,	AGCGGCGGTG			
26601	TGCTACTTTG	AATTGCCCGA	GGATCATATC	GAGGGCCCGG	CGCACGGCGT
	ACGATGAAAC	TTAACGGGCT	CCTAGTATAG	CTCCCGGGCC	GCGTGCCGCA
26651	CCGGCTTACC	GCCCAGGGAG	AGCTTGCCCG	TAGCCTGATT	CGGGAGTTTA
	GGCCGAATGG	CGGGTCCCTC	TCGAACGGGC	ATCGGACTAA	GCCCTCAAAT
26701		CCTGCTAGTT			
		GGACGATCAA			
26751		ACTGTCCTAA			
		TGACAGGATT	•		
26801		GAGTATAATA			
		CTCATATTAT			
26851		CTGTAAACGC			
•		GACATTTGCG			
26901		CCTGGTACTT			
		GGACCATGAA			
26951		AGACGGAGTG			
		TCTGCCTCAC			
27001	TACTCCATCA	GAAAAAACAC	CACCCTCCTT	ACCTGCCGGG	AACGTACGAG
		CTTTTTTGTG			
27051	TGCGTCACCG	GCCGCTGCAC	CACACCTACC	GCCTGACCGT	AAACCAGACT
		CGGCGACGTG			
27101	TTTTCCGGAC	AGACCTCAAT	AACTCTGTTT	ACCAGAACAG	GAGGTGAGCT
		TCTGGAGTTA			
27151		TTAGGGTATT			
	-	AATCCCATAA			
27201		AAGCAACTCT			
		TTCGTTGAGA		•	
27251	ATCGGGGTTG	GGGTTATTCT	CTGTCTTGTG	ATTCTCTTTA	TTCTTATACT
		CCCAATAAGA			
27301	AACGCTTCTC	TGCCTAAGGC	TCGCCGCCTG	CTGTGTGCAC	ATTTGCATTT
		ACGGATTCCG			
27351	ATTGTCAGCT	TTTTAAACGC	TGGGGTCGCC	ACCCAAGATG	ATTAGGTACA
		AAAATTTGCG		•	
27401	TAATCCTAGG	TTTACTCACC	CTTGCGTCAG	CCCACGGTAC	CACCCAAAAG
	ATTAGGATCC	AAATGAGTGG	GAACGCAGTC	GGGTGCCATG	@1.6661.1.1.1.C

Figure 27AC

27451	GTGGATTTTA CACCTAAAAT	A GCCAGC TCCTCGGTCG			
27501		ACTCTTATAA TGAGAATATT			
27551		AAACAAAATT TTTGTTTTAA			
27601		CTACAGAGTA GATGTCTCAT			
27651	TAAAACTTTT ATTTTGAAAA	ATGTATACTT TACATATGAA			
27701		CAAACAGTAT GTTTGTCATA			
27751	AACACTGGCA TTGTGACCGT	CTTTCTGCTG GAAAGACGAC			
27801		CTACTCTATA GATGAGATAT			
27851		AATGCCTTAA TTACGGAATT			
27901		ACTCGCTGCT TGAGCGACGA			
27951	AATTAGAATA TTAATCTTAT	GGATTTAAAC CCTAAATTTG			
28001		TTGACTCTAT AACTGAGATA			
28051		CCTGGATGTC GGACCTACAG			
28101		CCAGTCCAAC GGTCAGGTTG			
28151		9909900909 090990999	•		
28201					GCATGTGGTG CGTACACCAC
28251					TGGCTCATCT ACCGAGTAGA
28301	GCTGCCTAAA CGACGGATTT	GCGCAAACGC CGCGTTTGCG	GCCCGACCAC CGGGCTGGTG	CCATCTATAG GGTAGATATC	TCCCATCATT AGGGTAGTAA
28351	GTGCTACACC CACGATGTGG	CAAACAATGA GTTTGTTACT	TGGAATCCAT ACCTTAGGTA	AGATTGGACG TCTAACCTGC	GACTGAAACA CTGACTTTGT

Figure 27AD

28401	CATGTTCTTT	† TTACAG	TATGATTAAA	TGAGACATGÄ	TTCCTC T
	GTACAAGAAA	AGAGAATGTC	ATACTAATTT	ACTCTGTACT	AAGGAGCTCA
28451	TTTTATATTA	CTGACCCTTG	TTGCGCTTTT	TTGTGCGTGC	TCCACATTGG
	AAAATATAAT	GACTGGGAAC	AACGCGAAAA	AACACGCACG	AGGTGTAACC
28501	CTGCGGTTTC	TCACATCGAA	GTAGACTGCA	TTCCAGCCTT	CACAGTCTAT
	GACGCCAAAG	AGTGTAGCTT	CATCTGACGT	AAGGTCGGAA	GTGTCAGATA
28551	TTGCTTTACG	GATTTGTCAC	CCTCACGCTC	ATCTGCAGCC	TCATCACTGT
	AACGAAATGC	CTAAACAGTG	GGAGTGCGAG	TAGACGTCGG	AGTAGTGACA
28601	GGTCATCGCC	TTTATCCAGT	GCATTGACTG	GGTCTGTGTG	CGCTTTGCAT
	CCAGTAGCGG	AAATAGGTCA	CGTAACTGAC	CCAGACACAC	GCGAAACGTA
28651	ATCTCAGACA	CCATCCCCAG	TACAGGGACA	GGACTATAGC	TGAGCTTCTT
	TAGAGTCTGT	GGTAGGGGTC	ATGTCCCTGT	CCTGATATCG	ACTCGAAGAA
28701	AGAATTCTTT	AATTATGAAA	TTTACTGTGA	CTTTTCTGCT	GATTATTTGC
	TCTTAAGAAA	TIAATACTTT	AAATGACACT	GAAAAGACGA	CTAATAAACG
28751	ACCCTATCTG	CGTTTTGTTC	CCCGACCTCC	AAGCCTCAAA	GACATATATC
	TGGGATAGAC	GCAAAACAAG	GGGCTGGAGG	TTCGGAGTTT	CTGTATATAG
28801	ATGCAGATTC	ACTCGTATAT	GGAATATTCC	AAGTTGCTAC	AATGAAAAAA
	TACGTCTAAG	TGAGCATATA	CCTTATAAGG	TTCAACGATG	TTACTTTTTT
28851	GCGATCTTTC	CGAAGCCTGG	TTATATGCAA	TCATCTCTGT	TATGGTGTTC
	CGCTAGAAAG	GCTTCGGACC	AATATACGTT	AGTAGAGACA	ATACCACAAG
28901	TGCAGTACCA	TCTTAGCCCT	AGCTATATAT	CCCTACCTTG	ACATTGGCTG
	ACGTCATGGT	AGAATCGGGA	TCGATATATA	GGGATGGAAC	TGTAACCGAC
28951	GAACGCAATA	GATGCCATGA	ACCACCCAAC	TTTCCCCGCG	CCCGCTATGC
	CTTGCGTTAT	CTACGGTACT	TGGTGGGTTG	AAAGGGGCGC	GGGCGATACG
29001	TTCCACTGCA	ACAAGTTGTT	GCCGGCGGCT	TTGTCCCAGC	CAATCAGCCT
	AAGGTGACGT	TGTTCAACAA	CGGCCGCCGA	AACAGGGTCG	GTTAGTCGGA
29051	CGCCCACCTT	CTCCCACCC	CACTGAAATC	AGCTACTTTA	ATCTAACAGG
	GCGGGTGGAA	GAGGGTGGGG	GTGACTTTAG	TCGATGAAAT	TAGATTGTCC
29101	AGGAGATGAC	TGACACCCTA	GATCTAGAAA	TGGACGGAAT	TATTACAGAG
	TCCTCTACTG	ACTGTGGGAT	CTAGATCTTT	ACCTGCCTTA	ATAATGTCTC
29151	CAGCGCCTGC	TAGAAAGACG	CAGGGCAGCG	GCCGAGCAAC	AGCGCATGAA
	GTCGCGGACG	ATCTTTCTGC	GTCCCGTCGC	CGGCTCGTTG	TCGCGTACTT
29201	TCAAGAGCTC	CAAGACATGG	TTAACTTGCA	CCAGTGCAAA	AGGGGTATCT
	AGTTCTCGAG	GTTCTGTACC	AATTGAACGT	GGTCACGTTT	TCCCCATAGA
29251	TTTGTCTCGT	AAAGCAGGCC	AAAGTCACCT	ACGACAGTAA	TACCACCGGA
	AAACAGAGCA	TTTCGTCCGG	TTTCAGTGGA	TGCTGTCATT	ATGGTGGCCT
29301	CACCGCCTTA	GCTACAAGTT	GCCAACCAAG	CGTCAGAAAT	TGGTGGTCAT
	GTGGCGGAAT	CGATGTTCAA	CGGTTGGTTC	GCAGTCTTTA	ACCACCAGTA

Figure 27 AE

29351	GCTGGGAGAA CCACCCTCTT	A CCATTA TTCGGGTAAT	CCATAACTCA GGTATTGAGT	GCACTCGGTA CGTGAGCCAT	GAAACCTTC
29401	GCTGCATTCA	CTCACCTTGT	CAAGGACCTG	AGGATCTCTG	CACCCTTATT
				TCCTAGAGAC	
29451	AAGACCCTGT	GCGGTCTCAA	AGATCTTATT	CCCTTTAACT	AAAAAAAAA
	TTCTGGGACA	CGCCAGAGTT	TCTAGAATAA	GGGAAATTGA	TTATTTTTTT
29501	AATAATAAAG	CATCACTTAC	TTAAAATCAG	TTAGCAAATT	TCTGTCCAGT
	TTATTATTTC	GTAGTGAATG	AATTTTAGTC	AATCGTTTAA	AGACAGGTCA
29551	TTATTCAGCA	GCACCTCCTT	GCCCTCCTCC	CAGCTCTGGT	ATTGCAGCTT
	AATAAGTCGT	CGTGGAGGAA	CGGGAGGAGG	GTCGAGACCA	TAACGTCGAA
29601					TCAGTTTCCT
	GGAGGACCGA	CGTTTGAAAG	AGGTGTTAGA	TTTACCTTAC	AGTCAAAGGA
29651					GCAGATGAAG
		•		AGTACAACAA	
29701				CCCGTGTATC	
	GCGCGTTCTG	GCAGACTTCT	ATGGAAGTTG	GGGCACATAG	GTATACTGTG
29751	•••••			TACTCCTCCC	
					AAACATAGGG
29801					GCGCCTATCC
		•			CGCGGATAGG
29851					TGGGCAACGG
					ACCCGTTGCC
29901					GTAACCACTG
		•		•	CATTGGTGAC
29951					GGAAATATCT
					CCTTTATAGA
30001					CCGCCGCACC
					GGCGGCGTGG
30051					GCCCCGCTAA
					CGGGGCGATT
30101	CCGTGCACGA	CTCCAAACTT	AGCATTGCCA	CCCAAGGACC	CCTCACAGTG
					GGAGTGTCAC
30151	TCAGAAGGAA	ACCTACCCCT	GCAAACATCA	GCCCCCTCA	CCACCACCGA
					GGTGGTGGCT
30201					ACTGCCACTG
					TGACGGTGAC
30251	GTAGCTTGGG	CATTGACTTG	AAAGAGCCCA	TTTATACACA	AAATGGAAAA
	CATCGAACCC	GTAACTGAAC	TTTCTCGGGT	AAATATGTGT	TTTACCTTTT

Figure 27 AF

		1	mccmmncc » m	cm, s c s c s t c	ACCTAZ
30301	GATCCTGATT	A CGGGGC TCGCCCCG	AGGAAACGTA	CATTGTCTGC	TGGATTTOTG
30351	TTTGACCGTA AAACTGGCAT	GCAACTGGTC CGTTGACCAG			
30401		TACTGGAGCC ATGACCTCGG			
30451		CAGGAGGACT GTCCTCCTGA			
	ACTTGATGTT	AGTTATCCGT	TTGATGCTCA	AAACCAACTA	AATCTAAGAC
30551	TGAACTACAA	TCAATAGGCA			
30331	ATCCTGTCCC	GGGAGAAAA	TATTTGAGTC	GGGTGTTGAA	CCTATAATTG
30601		GCCTTTACTT CGGAAATGAA			
30651		CTAAGCACTG GATTCGTGAC			
30701		TGCAGGAGAT ACGTCCTCTA			
30751		CCCTCAAAAC GGGAGTTTTG			
30801	AAACAAGGCT	ATGGTTCCTA TACCAAGGAT	AACTAGGAAC	TGGCCTTAGT	TTTGACAGCA
30851	CAGGTGCCAT	TACAGTAGGA	AACAAAAATA	ATGATAAGCT	AACTTTGTGG
30901		ATGTCATCCT CTCCATCTCC			
30901	TGGTGTGGTC	GAGGTAGAGG	ATTGACATCT	GATTTACGTC	TCTTTCTACG
30951		TTGGTCTTAA AACCAGAATT			
31001		GGCTGTTAAA CCGACAATTT			
31051	CAAAGTGCTC GTTTCACGAG	ATCTTATTAT TAGAATAATA	AAGATTTGAC TTCTAAACTG	GAAAATGGAG CTTTTACCTC	TGCTACTAAA ACGATGATTT
31101	CAATTCCTTC GTTAAGGAAG	CTGGACCCAG GACCTGGGTC	AATATTGGAA TTATAACCTT	CTTTAGAAAT GAAATCTTTA	CCTCTAGAAT
31151	CTGAAGGCAC GACTTCCGTG	AGCCTATACA TCGGATATGT	AACGCTGTTG TTGCGACAAC	GATTTATGCC CTAAATACGG	TAACCTATCA ATTGGATAGT
31201	GCTTATCCAA CGAATAGGTT	AATCTCACGG TTAGAGTGCC	TAAAACTGCC ATTTTGACGG	AAAAGTAACA TTTTCATTGT	TTGTCAGTCA AACAGTCAGT

Figure 27 AB

31251	AGTTTACTTA TCAAATGAAT	AMEGGAGACA TTGCCTCTGT	AAACTAAACC TTTGATTTGG	TGTAACACTA ACATTGTGAT	ACCATTAGAC TGGTAATGTG
31301	TAAACGGTAC ATTTGCCATG	ACAGGAAACA TGTCCTTTGT	GGAGACACAA CCTCTGTGTT	CTCCAAGTGC GAGGTTCACG	ATACTCTATG TATGAGATAC
31351	TCATTTTCAT AGTAAAAGTA		TGGCCACAAC ACCGGTGTTG		
31401	CACATCCTCT GTGTAGGAGA	TACACTTTTT ATGTGAAAAA	CATACATTGC GTATGTAACG	CCAAGAATAA GGTTCTTATT	AGAATCGTTT TCTTAGCAAA
31451	GTGTTATGTT CACAATACAA		TATTTTTCAA ATAAAAAGTT		
31501		TCATCATATC	GGGGTGGTGG	TGTATCGAAT	ATGTCTAGTG
31551	GCATGGAATT	AGTTTGAGTG	AGAACCCTAG TCTTGGGATC	ATAAGTTGGA	CCCTCGAGGG
31601	AGGGTTGTGT	GTCTCATGTG	AGTCCTTTCT TCAGGAAAGA	GGGGCCGACC	GGAATTTTTC
31.651			ACATATTCTT TGTATAAGAA		
31701	TTTCCTGTCG AAAGGACAGC	AGCCAAACGC TCGGTTTGCG	TCATCAGTGA AGTAGTCACT	TATTAATAAA ATAATTATTT	CTCCCCGGGC GAGGGGCCCG
31751			GCTGTCCAGC CGACAGGTCG		
31801	TCCAACTTGC AGGTTGAACG	GGTTGCTTAA CCAACGAATT	CGGGCGGCGA	AGGAGAAGTC TCCTCTTCAG	CACGCCTACA GTGCGGATGT
31851	TGGGGGTAGA ACCCCCATCT	GTCATAATCG CAGTATTAGC	TGCATCAGGA ACGTAGTCCT	TAGGGCGGTG ATCCCGCCAC	GTGCTGCAGC CACGACGTCG
31901	AGCGCGCGAA TCGCGCGCTT	TAAACTGCTG ATTTGACGAC	GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	TCCGTCCTGC AGGCAGGACG	AGGAATACAA TCCTTATGTT
31951	CATGGCAGTG GTACCGTCAC	GTCTCCTCAG CAGAGGAGTC	CGATGATTCG GCTACTAAGC	CACCGCCCGC	AGCATAAGGC TCGTATTCCG
32001	GCCTTGTCCT CGGAACAGGA	CCGGGCACAG GGCCCGTGTC	CAGCGCACCC GTCGCGTGGG	TGATCTCACT ACTAGAGTGA	TAAATCAGCA ATTTAGTCGT
32051	CAGTAACTGC GTCATTGACG	AGCACAGCAC TCGTGTCGTG	CACAATATTG GTGTTATAAC	TTCAAAATCC	CACAGTGCAA GTGTCACGTT
32101	GGCGCTGTAT CCGCGACATA	CCAAAGCTCA GGTTTCGAGT	TGGCGGGGAC ACCGCCCCTG	CACAGAACCC GTGTCTTGGG	ACGTGGCCAT TGCACCGGTA
32151	CATACCACAA GTATGGTGTT	GCGCAGGTAG CGCGTCCATC	ATTAAGTGGC TAATTCACCG	GACCCCTCAT CTGGGGAGTA	AAACACGCTG TTTGTGCGAC

Figure 27 AH

32201					CCTCCC A GGAGGGCCAT
32251					ATCCTAAACC TAGGATTTGG
32301		AACCTGCCCG TTGGACGGGC			ACCGGGACTG TGGCCCTGAC
32351					TCATCATGCT AGTAGTACGA
32401		TCAATGTTGG AGTTACAACC			
32451		AAGCTCCTCC TTCGAGGAGG			
32501		TCAGCGTAAA AGTCGCATTT			CTCGCACGTA GAGCGTGCAT
32551					AGCGGATGAT TCGCCTACTA
32601		GGTAGCGCGG CCATCGCGCC			
32651		GAGTGCGCCG CTCACGCGGC			GTCGTAGTGT CAGCATCACA
32701		GGAACGCCGG CCTTGCGGCC			
32751		GACAAACAGA CTGITTGTCT			
32801		TAGTTGTAGT ATCAACATCA			
32851		GGGTTCTATG CCCAAGATAC			
32901		CCGCAGAATA GGCGTCTTAT			CACATTCGTT GTGTAAGCAA
32951					ACCATGTTTT TGGTACAAAA
	TTTTTTTATT AAAAAAATAA				
33051	GTGAACGCGC CACTTGCGCG	TCCCCTCCGG AGGGGAGGCC	TGGCGTGGTC ACCGCACCAG	AAACTCTACA TTTGAGATGT	GCCAAAGAAC CGGTTTCTTG
33101	AGATAATGGC TCTATTACCG				AAGGCAAACG TTCCGTTTGC

Figure 27 AI

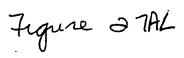
33151	GCCCTCACGT ( CGGGAGTGCA	GTCACCTG	GTAAAGGCTA CATTTCCGAT	AACCCTTCAG TTGGGAAGTC	TO TAKETOST
33201	CTCTATAAAC GAGATATTTG				
33251	GCCACCTTCT CGGTGGAAGA		CTAAGCAAAT GATTCGTTTA		
33301	ATTGTAAAAA TAACATTTTT		AGCGCCCTCC TCGCGGGAGG		
33351	AATCATGATT TTAGTACTAA		AGGTTCCTCA TCCAAGGAGT		
33401	AAGCGGAACA TTCGCCTTGT		TACCGCGATC ATGGCGCTAG		•
33451			AGGTCTGCAC TCCAGACGTG		
33501			AGAACCCACA TCTTGGGTGT		
33551	CGGAGCTATG GCCTCGATAC	CTAACCAGCG GATTGGTCGC	TAGCCCCGAT ATCGGGGCTA	GTAAGCTTGT CATTCGAACA	TGCATGGGCG ACGTACCCGC
33601			CTGCTCAAAA GACGAGTTTT		
33651	AAAAAAGAAA TTTTTTCTTT	GCACATCGTA CGTGTAGCAT	GTCATGCTCA CAGTACGAGT	TGCAGATAAA ACGTCTATTT	GGCAGGTAAG CCGTCCATTC
33701			AAGACACCAT TTCTGTGGTA		AACATGTCTG TTGTACAGAC
33751					ATTTAAACAT TAAATTTGTA
33801	TAGAAGCCTG ATCTTCGGAC	TCTTACAACA AGAATGTTGT	GGAAAAACAA CCTTTTTGTT	CCCTTATAAG GGGAATATTC	CATAAGACGG GTATTCTGCC
33851					CGTGATTAAA GCACTAATTT
33901	AAGCACCACC TTCGTGGTGG	GACAGCTCCT CTGTCGAGGA	CGGTCATGTC GCCAGTACAG	CGGAGTCATA GCCTCAGTAT	ATGTAAGACT TACATTCTGA
33951	CGGTAAACAC GCCATTTGTG	ATCAGGTTGA TAGTCCAACT	TTCACATCGG AAGTGTAGCC	TCAGTGCTAA AGTCACGATT	AAAGCGACCG TTTCGCTGGC
34001	AAATAGCCCG TTTATCGGGC	GGGGAATACA CCCCTTATGT	TACCCGCAGG ATGGGCGTCC	CGTAGAGACA GCATCTCTGT	ACATTACAGC TGTAATGTCG
34051	CCCCATAGGA GGGGTATCCT	GGTATAACAA CCATATTGTT	AATTAATAGG TTAATTATCC	AGAGAAAAAC TCTCTTTTTG	ACATAAACAC TGTATTTGTG

Figure 27AJ

34101	CTGAAAAACC GACTTTTTGG	TGCCTA GEACGGAT	GGCAAAATAG CCGTTTTATC	CACCCTCC@G GTGGGAGGGC	etecacata Gaggto
34151	ACATACAGCG TGTATGTCGC	CTTCCACAGC GAAGGTGTCG	GGCAGCCATA CCGTCGGTAT	ACAGTCAGCC TGTCAGTCGG	TTACCAGTAA AATGGTCATT
34201	AAAAGAAAAC TTTTCTTTTG	CTATTAAAAA GATAATTTT			
34251		TAAAAAAGGG ATTTTTTCCC			
34301	AAAAATGACG TTTTTACTGC	TAACGGTTAA ATTGCCAATT	AGTCCACAAA TCAGGTGTTT	AAACACCCAG TTTGTGGGTC	AAAACCGCAC TTTTGGCGTG
34351		GCCCAGAAAC CGGGTCTTTG			
34401		CGTTTTCCCA GCAAAAGGGT			
34451	ACAATTCCCA TGTTAAGGGT	ACACATACAA TGTGTATGTT	GTTACTCCGC CAATGAGGCG	CCTAAAACCT GGATTTTGGA	ACGTCACCCG TGCAGTGGGC
34501	CCCCGTTCCC GGGGCAAGGG	ACGCCCCGCG TGCGGGGGCGC	CCACGTCACA GGTGCAGTGT	AACTCCACCC TTGAGGTGGG	CCTCATTATC GGAGTAATAG
					PacI
34551		CAATCCAAAA GTTAGGTTTT			
34551 34601	TATAACCGAA		ATTCCATATA GGCTGGATGG	ATAACTACTA CCTTCCCCAT	CAATTAATTC TATGATTCTT
	TATAACCGAA  AATTCGGATC TTAAGCCTAG  CTCGCTTCCG	GTTAGGTTTT TGCGACGCGA	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG
34601	TATAACCGAA  AATTCGGATC TTAAGCCTAG  CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT	GTTAGGTTTT  TGCGACGCGA ACGCTGCGCT GCGGCATCGG	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA
34601 34651	TATAACCGAA  AATTCGGATC TTAAGCCTAG  CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA  GGAACCGTAA	GTTAGGTTTT  TGCGACGCGA ACGCTGCGCT  GCGGCATCGG CGCCGTAGCC  GACGACCATC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG	TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT
34601 34651 34701 34751	TATAACCGAA  AATTCGGATC TTAAGCCTAG  CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA GGAACCGTAA CCTTGGCATT	GTTAGGTTTT  TGCGACGCGA ACGCTGCGCT GCGCGTAGCC  GACGACCATC CTGCTGGTAG  AAAGGCCGCG TTTCCGGCGC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG
34601 34651 34701 34751 34801	TATAACCGAA  AATTCGGATC TTAAGCCTAG  CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA  GGAACCGTAA CCTTGGCATT  CCTGACGAGC GGACTGCTCG	GTTAGGTTTT  TGCGACGCGA ACGCTGCGCT GCGCGTAGCC  GACGACCATC CTGCTGGTAG  AAAGGCCGCG TTTCCGGCGC ATCACAAAAA TAGTGTTTTT  TAAAGATACC	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA TCGACGCTCA AGCTGCGAGT AGGCGTTCC	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG GGCGAAACCC CCGCTTTGGG
34601 34651 34701 34751 34801	TATAACCGAA  AATTCGGATC TTAAGCCTAG  CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA  CCTTGGCATT  CCTGACGAGC GGACTGCTCG GACAGGACTAC CCTGACGAGC GGACTGCTCG GACAGGACTA CTGTCCTGAT	GTTAGGTTTT  TGCGACGCGA ACGCTGCGCT  GCGGCATCGG CGCCGTAGCC  GACGACCATC CTGCTGGTAG  AAAGGCCGCG ATCACAAAAA TAGTGTTTTT  TAAAGATACC ATTTCTATGG  TCCGACCCTG	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA TCGACGCTCA AGCTGCGAGT AGGCGTTTCC TCCGCAAAGG CCGCTTACCG	ATAACTACTA CCTTCCCCAT GGAAGGGCTA TTGCAGGCCA AACGTCCGGT  TCAAGGCCAG AGTTCCGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC GGGACCTTCG GATACCTGTC	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCGCCCC CGAGGCGGGG GGCGAAACCC CCGCTTTGGG TCCCTCGTGC
34601 34651 34701 34751 34801 34851 34901	TATAACCGAA  AATTCGGATC TTAAGCCTAG  CTCGCTTCCG GAGCGAAGGC GCAGGTAGAT CGTCCATCTA  CCTTGGCATT  CCTGACGAGC GGACTGCTCG GACAGGACTAC CCTGACGAGC GGACTGCTCG GACAGGACTA CTGTCCTGAT	GTTAGGTTTT  TGCGACGCGA ACGCTGCGCTT GCGGCATCGG CGCCGTAGCC  GACGACCATC CTGCTGGTAG  AAAGGCCGCG ATCACAAAAA TAGTGTTTTT  TAAAGATACC ATTTCTATGG TCCGACCTG AGGCTGGGAC GCGTGGCGCT	ATTCCATATA GGCTGGATGG CCGACCTACC GATGCCCGCG CTACGGGCGC AGGGACAGCT TCCCTGTCGA TTGCTGGCGT AACGACCGCA AGCTGCGAGT AGCTGCGAGT CCGCAAAGG CCGCTTACCG GGCGAATGGC TTCTCATAGC	ATAACTACTA CCTTCCCCAT GGAAGGGGTA TTGCAGGCCA AACGTCCGGT TCAAGGCCAG AGTTCCGGTC TTTTCCATAG AAAAGGTATC AGTCAGAGGT TCAGTCTCCA CCCTGGAAGC GGGACCTTCG GATACCTGTC CTATGGACAG TCACGCTGTA	CAATTAATTC TATGATTCTT ATACTAAGAA TGCTGTCCAG ACGACAGGTC CAAAAGGCCA GTTTTCCGGT GCTCCGCCCC CGAGGCGGGG TCCCTCGTGC ACGGACACCC CGCTTTTCCG CGCCTTTCTC GCGGAAAGAG GGTATCTCAG

Figure 27 AK

35051	TTCAGCCCGA	CCGCTGCGCC	TTATCCGGTA	ACTATCGTCT	TGAGTCCÃAC
	AAGTCGGGCT	GGCGACGCGG	AATAGGCCAT	TGATAGCAGA	ACTCAGGTTG
35101	CCGGTAAGAC	ACGACTTATC	GCCACTGGCA	GCAGCCACTG	GTAACAGGAT
	GGCCATTCTG	TGCTGAATAG	CGGTGACCGT	CGTCGGTGAC	CATIGICCIA
35151	TAGCAGAGCG	አ ር ር ጥ አ ጥር ጥ አ ር	CCCCTCCTAC	<b>ል</b> ርልርጥፕረጥፕር	AAGTGGTGGC
32121	ATCGTCTCGC	TCCATACATC	CGCCACGATG	TCTCAAGAAC	TTCACCACCG
	A100101000				
35201	CTAACTACGG	CTACACTAGA	AGGACAGTAT	TTGGTATCTG	CGCTCTGCTG
	GATTGATGCC	GATGTGATCT	TCCTGTCATA	AACCATAGAC	GCGAGACGAC
				> CCMCM7C> M	CCCCCNNACN
35251	AAGCCAGTTA	CCTTCGGAAA	AAGAGTTGGT	MCCACAACTA	GGCCGTTTGT
	TICGGICAAT	GGAAGCCTTT	TICICANCCA	1CGAGARCIA	0000011101
35301	AACCACCGCT	GGTAGCGGTG	GTTTTTTTGT	TTGCAAGCAG	CAGATTACGC
JJ002	TTGGTGGCGA	CCATCGCCAC	CAAAAAAACA	AACGTTCGTC	GTCTAATGCG
35351	GCAGAAAAA	AGGATCTCAA	GAAGATCCTT	TGATCTTTTC	TACGGGGTCT
	CGTCTTTTTT	TCCTAGAGTT	CTTCTAGGAA	ACTAGAAAAG	ATGUCCUAGA
25.401	GACGCTCAGT	CC22CC2222	ርብረ ሃርርብብ ን	ದದ್ದು ಸಿಗಿಗಾಗದ್ದಿದ್ದ	TCATGAGATT
35401	CTCCCACTCA	CCALCCARA	GAGTGCAATT	CCCTAAAACC	AGTACTCTAA
35451	ATCAAAAAGG	ATCTTCACCT	AGATCCTTTT	AAATCAATCT	AAAGTATATA
	TAGTTTTTCC	TAGAAGTGGA	TCTAGGAAAA	TTTAGTTAGA	TTTCATATAT
					0100010000
35501	TGAGTAAACT	TGGTCTGACA	GTTACCAATG CAATGGTTAC	CTTAATCAGT	GAGGCACCTA
	ACTCATTTGA	ACCAGACTGT	CAATGGTTAC	GAATTAGICA	CICCGIGGAI
35551	TOTOLGOGAT	ርጥርጥርጥልጥጥ	CGTTCATCCA	TAGTTGCCTG	ACTCCCCGTC
33331	AGAGTCGCTA	GACAGATAAA	GCAAGTAGGT	ATCAACGGAC	TGAGGGGCAG
35601	GTGTAGATAA	CTACGATACG	GGAGGGCTTA	CCATCTGGCC	CCAGTGCTGC
	CACATCTATT	GATGCTATGC	CCTCCCGAAT	GGTAGACCGG	GGTCACGACG
		CCACACCCAC	<b>ででかてなてでほご</b> て	ጥ/ ጉርኔ ጥጥባል	TCAGCAATAA
35651	MATGATACCG	CCACACCCAC	CGAGTGGCCG	AGGTCTAAAT	AGTCGTTATT
	TIACIAIGGC	0010100010	00		
35701	ACCAGCCAGC	CGGAAGGGCC	GAGCGCAGAA	GTGGTCCTGC	AACTTTATCC
	TGGTCGGTCG	GCCTTCCCGG	CTCGCGTCTT	CACCAGGACG	TTGAAATAGG
					. m om. om.
35751	GCCTCCATCC	AGTCTATTAA	TIGTIGCCGG	GAAGCTAGAG	TAAGTAGTTC ATTCATCAAG
	CGGAGGTAGG	TCAGATAATT	AACAACGGCC	CITCGATCIC	AIICAICAAG
25001	<b>ር</b> ርር አርጥጥ አልጥ	ACTITICCGCA	ACGTTGTTGC	CATTGCTACA	GGCATCGTGG
33601	CGGTCAATTA	TCAAACGCGT	TGCAACAACG	GTAACGATGT	CCGTAGCACC
35851	TGTCACGCTC	GTCGTTTGGT	ATGGCTTCAT	TCAGCTCCGG	TTCCCAACGA
	ACAGTGCGAG	CAGCAAACCA	. TACCGAAGTA	AGTCGAGGCC	AAGGGTTGCT
		mm1 ^1 m^1 c	·	. ጥርሮአአአአአ	<b>רוביוו</b> יז בוריווי
35901	TCAAGGCGAG	TIACATGATC	CCCCATGITC	· TOCUMENTAL	CGGTTAGCTC CCCAATCGAG
	AGTICUGUTU	MUTGINCING	GOGINCAN		
35951	CTTCGGTCCT	CCGATCGTTG	TCAGAAGTAA	GTTGGCCGC	GTGTTATCAC
<b></b>	GAAGCCAGGA	GGCTAGCAAC	AGTCTTCATT	CAACCGGCG	CACAATAGIG



WO 02/22080 PCT/US01/28861 36001 TCATGGTTAT SAAGCACTG CATAATTCTC TTACTGTCAT GCCATCATA AGTACCAATA CCGTCGTGAC GTATTAAGAG AATGACAGTA CGGTAGGCAT 36051 AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT TCTGAGAATA TCTACGAAAA GACACTGACC ACTCATGAGT TGGTTCAGTA AGACTCTTAT 36101 GTGTATGCGG CGACCGAGTT GCTCTTGCCC GGCGTCAACA CGGGATAATA CACATACGCC GCTGGCTCAA CGAGAACGGG CCGCAGTTGT GCCCTATTAT 36151 CCGCGCCACA TAGCAGAACT TTAAAAGTGC TCATCATTGG AAAACGTTCT GGCGCGGTGT ATCGTCTTGA AATTTTCACG AGTAGTAACC TTTTGCAAGA 36201 TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT AGCCCCGCTT TTGAGAGTTC CTAGAATGGC GACAACTCTA GGTCAAGCTA 36251 GTAACCCACT CGTGCACCCA ACTGATCTTC AGCATCTTTT ACTTTCACCA CATTGGGTGA GCACGTGGGT TGACTAGAAG TCGTAGAAAA TGAAAGTGGT 36301 GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC AAAAAAGGGA CGCAAAGACC CACTCGTTTT TGTCCTTCCG TTTTACGGCG TTTTTTCCCT 36351 ATAAGGGCGA CACGGAAATG TTGAATACTC ATACTCTTCC TTTTTCAATA TATTCCCGCT GTGCCTTTAC AACTTATGAG TATGAGAAGG AAAAAGTTAT 36401 TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTTG

AATAACTTCG TAAATAGTCC CAATAACAGA GTACTCGCCT ATGTATAAAC

TTACATAAAT CTTTTTATTT GTTTATCCCC AAGGCGCGTG TAAAGGGGCT

ATTTTTATCC GCATAGTGCT CCGGGAAAGC AGAAGTTCTT AACCTAGGCT

36451 AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA

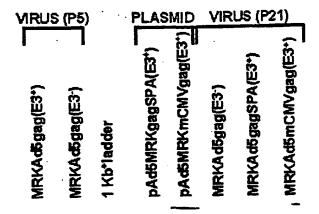
36501 AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTAACCTA
TTTCACGGTG GACTGCAGAT TCTTTGGTAA TAATAGTACT GTAATTGGAT

36551 TAAAAATAGG CGTATCACGA GGCCCTTTCG TCTTCAAGAA TTGGATCCGA

## PacI

36601 ATTCTTAATT TCTTAATTAA (SEQ ID NO:34)
TAAGAATTAA AGAATTAATT (SEQ ID NO:35)

Figure 27AM



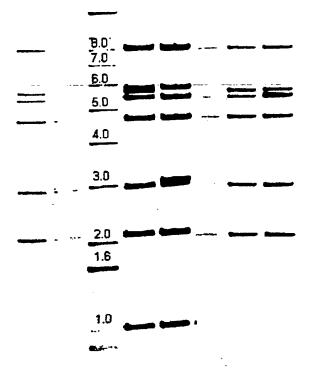


FIGURE 28

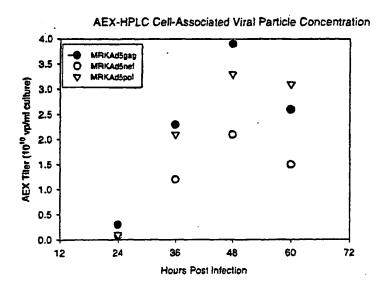


FIGURE 29A

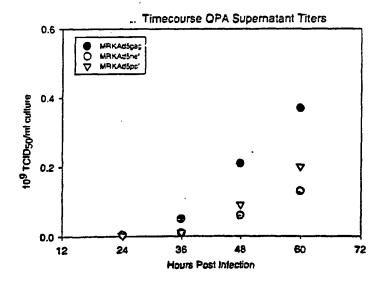


FIGURE 29B

PCT/US01/28861 WO 02/22080

atg Met 1	gat Asp	gca Ala	atg Met	aag Lys 5	aga Arg	ggg ggg	ctc Leu	tgc Cys	tgt Cys 10	gtg Val	ctg Leu	ctg Leu	ctg Leu	tgt Cys 15	gga Gly	48
gca Ala	gtc Val	ttc Phe	gtt Val 20	tcg Ser	ccc Pro	agc Ser	gag Glu	atc Ile 25	tcc Ser	att Ile	gtg Val	tgg Trp	gcc Ala 30	tcc Ser	agg Arg	96
gag Glu	ctg Leu	gag Glu 35	agg Arg	ttt Phe	gct Ala	gtg Val	aac Asn 40	cct Pro	ggc Gly	ctg Leu	ctg Leu	gag Glu 45	acc Thr	tct Ser	gag Glu	144
GJA BBB	tgc Cys 50	agg Arg	cag Gln	atc Ile	ctg Leu	ggc Gly 55	cag Gln	ctc Leu	cag Gln	ccc Pro	tcc Ser 60	ctg Leu	caa Gln	aca Thr	Gly	192
tct Ser 65	gag Glu	gag Glu	ctg Leu	agg Arg	tcc Ser 70	ctg Leu	tac Tyr	aac Asn	aca Thr	gtg Val 75	gct Ala	acc Thr	ctg Leu	tac Tyr	tgt Cys 80	240
gtg Val	cac His	cag Gln	aag Lys	att Ile 85	gat Asp	gtg Val	aag Lys	gac Asp	acc Thr 90	aag Lys	gag Glu	gcc Ala	ctg Leu	gag Glu 95	aag Lys	288
att Ile	gag Glu	gag Glu	gag Glu 100	cag Gln	aac Asn	aag Lys	tcc Ser	aag Lys 105	aag Lys	aag Lys	gcc Ala	cag Gln	cag Gln 110	gct Ala	gct Ala	336
gct Ala	ggc Gly	aca Thr 115	ggc Gly	aac Asn	tcc Ser	agc Ser	cag Gln 120	gtg Val	tcc Ser	cag Gln	aac Asn	tac Tyr 125	ccc Pro	att Ile	gtg Val	384
cag Gln	aac Asn 130	ctc Leu	cag Gln	ggc	cag Gln	atg Met 135	gtg Val	cac His	cag Gln	gcc Ala	atc Ile 140	tcc Ser	ccc Pro	cgg Arg	acc Thr	432
ctg Leu 145	aat Asn	gcc Ala	tgg Trp	gtg Val	aag Lys 150	gtg Val	gtg Val	gag Glu	gag Glu	aag Lys 155	gcc Ala	ttc Phe	tcc Ser	cct Pro	gag Glu 160	480
gtg Val	atc Ile	ccc Pro	atg Met	ttc Phe 165	tct Ser	gcc Ala	ctg Leu	tct Ser	gag Glu 170	ggt Gly	gcc Ala	acc Thr	ccc Pro	cag Gln 175	gac Asp	528
ctg Leu	aac Asn	acc Thr	atg Met 180	ctg Leu	aac Asn	aca Thr	gtg Val	ggg Gly 185	GIA	cat His	cag Gln	gct Ala	gcc Ala 190	atg Met	cag Gln	576
atg Met	ctg Leu	aag Lys 195	Glu	acc Thr	atc	aat Asn	gag Glu 200	Glu	gct Ala	gct Ala	gag Glu	tgg Trp 205	Asp	agg Arg	ctg Leu	624
cat His	cct Pro 210	Val	cac	gct Ala	ggc	Pro 215	Ile	gcc Ala	ccc Pro	ggc	Gln 220	Met	agg Arg	gag Glu	Pro	672
agg Arg 225	Gly	tct Ser	gac Asp	att Ile	gct Ala 230	GLY	acc Thr	acc	tcc Ser	acc Thr 235	Let	cag Gln	gag Glu	cag Glr	att Ile 240	720
ggc Gly	tgg Trp	atg Met	acc	aac Asr 245	Asn	ecc Pro	ccc Pro	ato Ile	cct Pro 250	va1	ggg Gly	g gaa / Glu	ato Ile	tac Tyr 255	aag Lys	768

Figure 30 A\*

agg Arg	tgg Trp	atc Ile	atc Ile 260	ctg Leu	ggc	ctg Leu	aac Asn	aag Lys 265	att Ile	gtg Val	agg Arg	atg Met	tac Tyr 270	tcc Ser	ccc Pro	816
acc Thr	tcc Ser	atc Ile 275	ctg Leu	gac Asp	atc Ile	agg Arg	cag Gln 280	ggc Gly	ccc Pro	aag Lys	gag Glu	ccc Pro 285	ttc Phe	agg Arg	gac Asp	864
tat Tyr	gtg Val 290	gac Asp	agg Arg	ttc Phe	tac Tyr	aag Lys 295	acc Thr	ctg Leu	agg Arg	gct Ala	gag Glu 300	cag Gln	gcc Ala	tċc Ser	cag Gln	912
gag Glu 305	gtg Val	aag Lys	aac Asn	tgg Trp	atg Met 310	aca Thr	gag Glu	acc Thr	ctg Leu	ctg Leu 315	gtg Val	cag Gln	aat Asn	gcc Ala	aac Asn 320	960
cct Pro	gac Asp	tgc Cys	aag Lys	acc Thr 325	atc Ile	ctg Leu	aag Lys	gcc Ala	ctg Leu 330	ggc Gly	ect Pro	gct Ala	gcc Ala	acc Thr 335	ctg Leu	1008
gag Glu	gag Glu	atg Met	atg Met 340	aca Thr	gcc Ala	tgc Cys	cag Gln	ggg Gly 345	gtg Val	eja aaa	ggc	ect Pro	ggt Gly 350	cac His	aag Lys	1056
gcc Ala	agg Arg	gtg Val 355	ctg Leu	gct Ala	gag Glu	gcc Ala	atg Met 360	tcc Ser	cag Gln	gtg Val	acc Thr	aac Asn 365	tcc Ser	gcc Ala	acc Thr	1104
atc Ile	atg Met 370	atg Met	cag Gln	agg Arg	ggc Gly	aac Asn 375	ttc Phe	agg Arg	aac Asn	cag Gln	agg Arg 380	aag Lys	aca Thr	gtg Val	aag Lys	1152
tgc Cys 385	ttc Phe	aac Asn	tgt Cys	ggc Gly	aag Lys 390	gtg Val	ggc Gly	cac His	att Ile	gcc Ala 395	aag Lys	aac Asn	tgt Cys	agg Arg	gcc Ala 400	1200
ccc Pro	agg Arg	aag Lys	aag Lys	ggc Gly 405	Cys	tgg Trp	aag Lys	tgt Cys	ggc Gly 410	aag Lys	gag Glu	ggc Gly	cac His	cag Gln 415	atg Met	1248
aag Lys	gac Asp	tgc Cys	aat Asn 420	gag Glu	agg Arg	cag Gln	gcc Ala	aac Asn 425	ttc Phe	ctg Leu	GJ7 ggc	aaa Lys	atc Ile 430	tgg Trp	ccc Pro	1296
tcc Ser	cac His	aag Lys 435	ggc Gly	agg Arg	cct Pro	ggc Gly	aac Asn 440	ttc Phe	ctc Leu	cag Gln	tcc Ser	agg Arg 445	cct Pro	gag Glu	ccc Pro	1344
Thr	gcc Ala 450	Pro	ccc Pro	gag Glu	gag Glu	tcc Ser 455	ttc Phe	agg Arg	ttť Phe	GJÀ âââ	gag Glu 460	gag Glu	aag Lys	acc Thr	acc Thr	1392
ccc Pro 465	agc Ser	cag Gln	aag Lys	cag Gln	gag Glu 470	ccc Pro	att Ile	gac Asp	aag Lys	gag Glu 475	ctg Leu	tac Tyr	ccc Pro	ctg Leu	gcc Ala 480	1440
tcc Ser	ctg Leu	agg Arg	tcc Ser	ctg Leu 485	ttt Phe	ggc Gly	aac Asn	gac Asp	ccc Pro 490	tcc Ser	tcc Ser	cag Gln	taa *	(SII	NO:36) NO:37)	1482

Figure 30 B

Figure 31

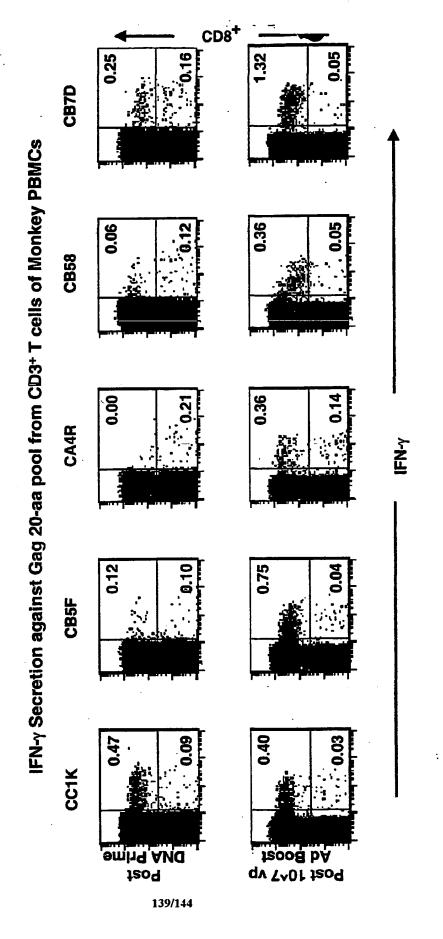
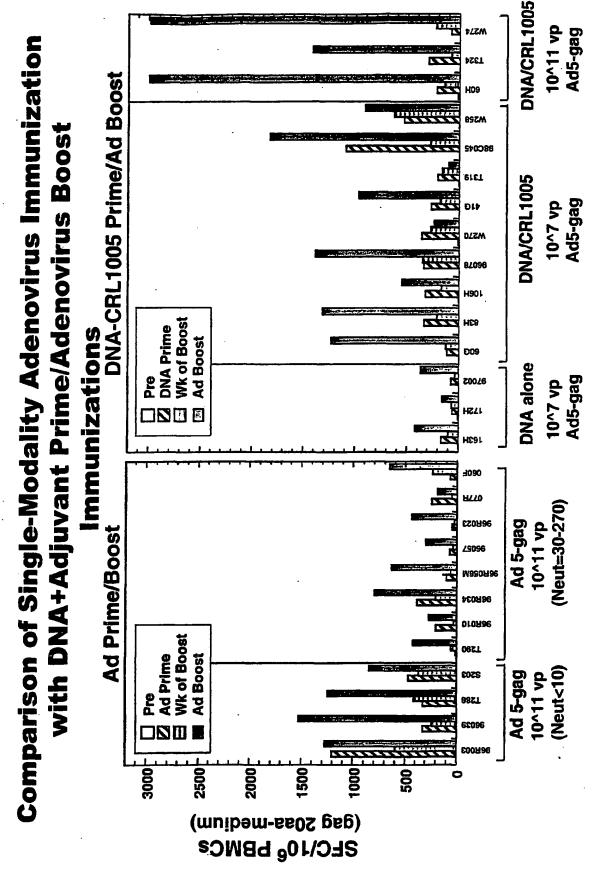


FIGURE 52



# FIGURE 33A

ATGGGTGCTA	GGGCTTCTGT	GCTGTCTGGT	GGTGAGCTGG	ACAAGTGGGA	GAAGATCAGG
CTGAGGCCTG	GTGGCAAGAA	GAAGTACAAG	CTAAAGCACA	TTGTGTGGGC	CTCCAGGGAG
CTGGAGAGGT	TTGCTGTGAA	CCCTGGCCTG	CTGGAGACCT	CTGAGGGGTG	CAGGCAGATC
CTGGGCCAGC	TCCAGCCCTC	CCTGCAAACA	GGCTCTGAGG	AGCTGAGGTC	CCTGTACAAC
ACAGTGGCTA	CCCTGTACTG	TGTGCACCAG	AAGATTGATG	TGAAGGACAC	CAAGGAGGCC
CTGGAGAAGA	TTGAGGAGGA	GCAGAACAAG	TCCAAGAAGA	AGGCCCAGCA	GGCTGCTGCT
GGCACAGGCA	ACTCCAGCCA	GGTGTCCCAG	AACTACCCCA	TTGTGCAGAA	CCTCCAGGGC
CAGATGGTGC	ACCAGGCCAT	CTCCCCCGG	ACCCTGAATG	CCTGGGTGAA	GGTGGTGGAG
GAGAAGGCCT	TCTCCCCTGA	GGTGATCCCC	ATGTTCTCTG	${\tt CCCTGTCTGA}$	GGGTGCCACC
CCCCAGGACC	TGAACACCAT	GCTGAACACA	GTGGGGGGCC	ATCAGGCTGC	CATGCAGATG
CTGAAGGAGA	CCATCAATGA	GGAGGCTGCT	GAGTGGGACA	GGCTGCATCC	TGTGCACGCT
GGCCCCATTG	CCCCGGCCA	GATGAGGGAG	CCCAGGGGCT	CTGACATTGC	TGGCACCACC
TCCACCCTCC	AGGAGCAGAT	TGGCTGGATG	ACCAACAACC	CCCCCATCCC	TGTGGGGGAA
	GGTGGATCAT				
	ACATCAGGCA				
TACAAGACCC	TGAGGGCTGA	GCAGGCCTCC	CAGGAGGTGA	AGAACTGGAT	GACAGAGACC
	AGAATGCCAA				
GCCACCCTGG	AGGAGATGAT	GACAGCCTGC	CAGGGGGTGG	GGGGCCCTGG	TCACAAGGCC
AGGGTGCTGG	CTGAGGCCAT	GTCCCAGGTG	ACCAACTCCG	CCACCATCAT	GATGCAGAGG
GGCAACTTCA	GGAACCAGAG	GAAGACAGTG	AAGTGCTTCA	ACTGTGGCAA	GGTGGGCCAC
ATTGCCAAGA	ACTGTAGGGC	CCCCAGGAAG	AAGGGCTGCT	GGAAGTGTGG	CAAGGAGGC
CACCAGATGA	AGGACTGCAA	TGAGAGGCAG	GCCAACTTCC	TGGGCAAAAT	CTGGCCCTCC
CACAAGGGCA	GGCCTGGCAA	CTTCCTCCAG	TCCAGGCCTG	AGCCCACAGC	CCCTCCCGAG
	GGTTTGGGGA				
AAGGAGCTGT	ACCCCCTGGC	CTCCCTGAGG	TCCCTGTTTG	GCAACGACCC	CTCCTCCCAG
ATGGCTCCCA	TCTCCCCCAT	TGAGACTGTG	CCTGTGAAGC	TGAAGCCTGG	CATGGATGGC
	AGCAGTGGCC				
	AGAAGGAGGG				
					GGACTTCAGG
					CCACCCCGCT
					CTTCTCTGTG
					CAACAATGAG
					CTCCCCTGCC
					CCCTGACATT
					TGGGCAGCAC
					CACCCTGAC
					CCCCGACAAG
					TGACATCCAG
					GGTGAGGCAG
					GACTGAGGAG
GCTGAGCTGG	AGCTGGCTGA	GAACAGGGAG	ATCCTGAAGG	AGCCTGTGCA	TGGGGTGTAC

## FIGURE 33B

TATGACCCCT CCAAGGACCT GATTGCTGAG ATCCAGAAGC AGGGCCAGGG CCAGTGGACC TACCAAATCT ACCAGGAGCC CTTCAAGAAC CTGAAGACTG GCAAGTATGC CAGGATGAGG GGGGCCCACA CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG TCCATTGTGA TCTGGGGCAA GACCCCCAAG TTCAAGCTGC CCATCCAGAA GGAGACCTGG GAGACCTGGT GGACTGAGTA CTGGCAGGCC ACCTGGATCC CTGAGTGGGA GTTTGTGAAC ACCCCCCCC TGGTGAAGCT GTGGTACCAG CTGGAGAAGG AGCCCATTGT GGGGGCTGAG ACCITCTATG TGGCTGGGGC TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG ACCAACAGGG GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC CTCCAGGCCA TCTACCTGGC CCTCCAGGAC TCTGGCCTGG AGGTGAACAT TGTGACTGCC TCCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC AGTCTGAGTC TGAGCTGGTG AACCAGATCA TTGAGCAGCT GATCAAGAAG GAGAAGGTGT ACCTGGCCTG GGTGCCTGCC CACAAGGGCA TTGGGGGCAA TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG GTGCTGTTCC TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCCTGTGG TGGCTAAGGA GATTGTGGCC TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG GGCAGGTGGA CTGCTCCCCT GGCATCTGGC AGCTGGCCTG CACCCACCTG GAGGGCAAGG TGATCCTGGT GGCTGTGCAT GTGGCCTCCG GCTACATTGA GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC TACTTCCTGC TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC TCCAACTTCA CTGGGGCCAC AGTGAGGGCT GCCTGCTGGT GGGCTGGCAT CAAGCAGGAG TTTGGCATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG CCTCCATGAA CAAGGAGCTG AAGAAGATCA TTGGGCAGGT GAGGGACCAG GCTGAGCACC TGAAGACAGC TGTGCAGATG GCTGTGTTCA TCCACAACTT CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG AGGATTGTGG ACATCATTGC CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCCTGTG GAAGGGCCCT GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC AGGACAACTC TGACATCAAG GTGGTGCCCA GGAGGAAGGC CAAGATCATC AGGGACTATG GCAAGCAGAT GGCTGGGGAT GACTGTGTGG CCTCCAGGCA GGATGAGGAC TAA SEO ID NO: 38

142/144

#### FIGURE 34A

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Ala Gly Thr Gly Asn Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Asn Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu Lys Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro

## FIGURE 34B

Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Jle Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp SEQ ID NO: 39

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The formal (uspro)

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